



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 33/26, 33/06, 33/24	A1	(11) International Publication Number: WO 99/15189 (43) International Publication Date: 1 April 1999 (01.04.99)
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(54) Title: METAL COMPOUNDS, MIXED OR SULPHATED, AS PHOSPHATE BINDERS		
(57) Abstract A mixed metal compound for pharmaceutical use is free from aluminium and has a phosphate binding capacity of at least 30 %, by weight of the total weight of phosphate present, over a pH range of from 2-8. The compound is especially useful for treatment of hyperphosphataemia. The metals are preferably iron (III) and at least one of calcium, magnesium, lanthanum and cerium. A metal sulphate for pharmaceutical use is selected from at least one of calcium, lanthanum and cerium sulphate compounds and has a phosphate binding capacity of at least 30 % by weight of the total phosphate present, over a pH range from 2-8.		

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METAL COMPOUNDS, MIXED OR SULPHATED, AS PHOSPHATE BINDERS

This invention relates to metal compounds, especially metal compounds free from aluminium, for pharmaceutical application, especially as phosphate binders.

5 WO-A-94/09798 discloses mixtures or complexes containing calcium and sulphate for use in a wide variety of pharmaceutical applications. The mixtures or complexes are inorganic compositions derivable from peat, in the form of aqueous solutions or synthetic syngenite ($\text{CaSO}_4 \cdot \text{K}_2\text{SO}_4 \cdot \text{H}_2\text{O}$) materials. There is no reference to their
10 phosphate binding capacity.

In patients with kidney failure on haemodialysis (of whom there are 6,000,000 world wide), phosphate concentrations in the blood plasma can rise dramatically and such hyperphosphataemia can result in calcium phosphate deposition in soft tissue. Currently, the plasma phosphate
15 levels are reduced by oral intake of inorganic and organic phosphate binders. The most common treatment in the UK is with aluminium hydroxide gel ("Aludrox" at 4g/day) which forms an insoluble aluminium phosphate. However, this results in further toxic complications due to Al accumulation, eg reduction in haemoglobin production, impairment in
20 natural repair and production of bone and possible impairment of neurological/cognitive function. Improvements in phosphate binding capacity as compared with aluminium hydroxide gel have been achieved with other aluminium compounds such as microcrystalline aluminium oxide hydroxide (boehmite) and certain hydrotalcites have been made;
25 Ookubo et al, Journal Pharmaceutical Sciences (November 1992), **81(11)**,1139-1140. However, such compounds still result in an intolerable amount of aluminium accumulation in renal failure patients. It is also known to use calcium compounds having poor solubility at pH 6-9, eg calcium carbonate, hydroxide, oxide and/or sulphate in a medicinal form

resistant to gastric juices. However, it is known that, for example, with calcium carbonate, a large dosage is required because of its relatively low in vivo capacity for phosphate removal, such large dosages also being difficult to administer. This can cause further complications associated with high calcium intake. It has also been proposed (WO-A-92/01458) to control serum phosphate levels in patients suffering from or predisposed to hyperphosphataemia by contacting ingested phosphate with an oxy-iron compound selected from ferric oxides, oxy-hydroxides and hydroxides. Similarly, Spengler et al, Nephrol. Dial. Transplant. (1996), 11, 808-812, suggests treatment of hyperphosphataemia with a complex of iron (III) oxide-hydroxide modified dextran. However, in the tests conducted, extremely high dosage amounts to animals were given. Moreover, many inorganic preparations are efficient phosphate binders only over a limited pH range, especially an acid pH range of about 3-5. Such current phosphate binders effective at pH3 would not necessarily bind as effectively at higher pH, eg ≥ 7 , which obtain in the lower tract, eg duodenum and below, and where at least some of the binding of phosphate may take place. Moreover, particularly alkaline binders could buffer the stomach pH up to a high level at which they would not have a phosphate binding capacity.

Thus, there is an urgent and widespread need for a phosphate binder which does not release aluminium into the blood stream, which does not provide long term side effects, which can be administered in relatively low dosages and which is effective over a wide pH range of from say 2-8.

We have found surprisingly that certain mixed metal compounds, which are free from aluminium, may bind at least 30% by weight of the total weight of phosphate present over a pH range of from 2-8.

Thus, according to a first aspect, the invention provides a mixed metal compound for pharmaceutical use which is free from aluminium and

which has a phosphate binding capacity of at least 30%, by weight of the total weight of phosphate present, over a pH range of from 2-8.

According to a second aspect, the invention provides the use, in the preparation of a medicament for treating hyperphosphataemia, of a mixed metal compound free from aluminium and having a phosphate binding capacity of at least 30%, by weight of the total weight of phosphate present, over a pH range of from 2-8.

Such mixed metal compounds may contain iron (III) and at least one of magnesium, calcium, lanthanum and cerium.

Preferably the mixed metal compounds contain at least one of hydroxyl and carbonate anions and optionally additionally, at least one of sulphate, chloride and oxide.

It is believed that preferred mixed metal hydroxy carbonates containing each of magnesium and iron are of a hydrotalcite structure.

For such mixed metal compounds, it is generally preferable to use unaged hydrotalcites, which have not been subjected to a drying operation.

However, it is even more preferable to use mixed calcium/ferric mixed metal compound which seem to be equally effective whether unaged or not.

Even more preferably, the ratio of Ca^{2+} : Fe^{3+} is at least 2:1., still more preferably at least 3:1.

An alternative preferred compound contains Ca^{2+} , Mg^{2+} and Fe^{3+} , more preferably in a ratio of 3:3:2.

Further investigation of calcium rich compounds led us to find that although anhydrous calcium sulphate as such is a poor phosphate binder, after treatment of calcium sulphate, for example, anhydrous calcium sulphate, with an alkaline material, it became an extremely effective phosphate binder. This result is particularly surprising.

We predict also that each of lanthanum and cerium sulphate will behave similarly.

Thus, according to another aspect, the invention provides metal sulphate material for pharmaceutical use, which metal sulphate material is selected from at least one of calcium, lanthanum and cerium sulphate compounds treated with an alkali solution, preferably an aqueous solution of an alkaline hydroxide, more preferably sodium hydroxide, which said material comprises a solid material, especially a solid material or a suspension of a solid material in a liquid especially aqueous, medium.

According to a further aspect of the invention there is provided the use in a method of preparing a medicament for treatment of hyperphosphataemia of a metal sulphate material selected from at least one of calcium, lanthanum and cerium sulphate compounds treated with an alkali solution.

According to a still further aspect, there is provided a method of preparing a metal sulphate material, which method comprises treating a metal sulphate selected from at least one of calcium, lanthanum and cerium sulphate with an alkali solution.

Preferred embodiments of the invention will now be described in more detail with reference to the following Examples (which also include comparative tests) and graphical representations. In each of Figs. 1 - 8, the ordinate (y-axis) gives the percentage of phosphate bound and the abscissa (x-axis) the pH. In the Figures,

Fig. 1 shows the effect of pH and ageing on percentage phosphate binding of mixed metal compounds. In Fig.1,

25	○	Mg:Fe	3:1	prep 2 unaged
	●	Mg:Fe	3:1	prep 2 unaged
	△	Mg:Fe	2:1	prep 1 unaged
	▲	Mg:Fe	2:1	prep 1 aged
	□	Ca:Fe	3:1	unaged
	■	Ca:Fe	3:1	aged
30	★	Ca:Fe:Mg		unaged

★ Ca:Mg:Fe aged

Fig.2 shows the effect of pH and drying on percentage phosphate binding of mixed metal compounds. In Fig. 2,

5	○	Mg:Fe	3:1	prep 3 wet
	●	Mg:Fe	3:1	prep 3 dry
	△	Mg:Fe	2:1	prep 2 wet
10	▲	Mg:Fe	3:1	prep 2 dry
	□	Ca:Fe	3:1	wet
	■	Ca:Fe	3:1	dry
	★	Ca:Fe:Mg		wet
	★	Ca:Mg:Fe		dry

Fig. 3 shows the effect of increasing weight of compound on percentage phosphate bound at pH3. In Fig. 3,

15	▲-▲	Mg(OH) ₂	
	△-△	Mg:Fe 2:1	Prep 1 unaged wet
	○-○	CT100	
20	■-■	CaFe 3:1	Aged wet
	●-●	Altacite liquid washed	
	★-★	Al(OH) ₃	

Fig. 4 shows the effect of increasing weight of compound on percentage phosphate bound at pH7. In Fig. 4,

25	■-■	CaFe 3:1	Aged wet
	○-○	CT100	
	●-●	Altacite liquid washed	
	★-★	Al(OH) ₃	

Fig. 5 shows the time course of phosphate binding in food. In Fig.

5,	○	Al(OH) ₃	
	□	CT Fe:Mg	2:1 unaged unwashed
	▲	Ce(OH) ₃	

- ▼ Altacite liquid unwashed
- ◇ $\text{Mg}(\text{OH})_2$
- ★ Milk of magnesia ($1.8\text{g Mg}(\text{OH})_3$)
- ★ CT100 washed

5 Fig. 6 shows the effect of phosphate binding by the calcium ferric iron preparations over the pH range 3-8. In Fig. 6,

- Ca:Fe 1:1 ratio
- Δ-Δ Ca:Fe 2:1 ratio
- Ca:Fe 3:1 ratio prep 1
- 10 ●-● Ca:Fe 3:1 ratio prep 2
- ▽-▽ Ca:Fe 5:1 ratio
- ★-★ Ca:Fe 3:1 ratio (from chloride salts)
- ▼-▼ Ca:Fe 3:1 ratio (with prior ppt of metals)

15 Fig. 7 shows the effect of phosphate binding by the magnesium ferric iron and calcium magnesium ferric iron preparations over the pH range 3-8. In Fig. 7,

- Δ-Δ Mg:Fe 2:1 Prep 1
- ▲-▲ Mg:Fe 2:1 Prep 2
- Mg:Fe 3:1 Prep 1
- 20 ●-● Mg:Fe 3:1 Prep 2
- Mg:Fe 3:1 Prep 3
- Mg:Fe 3:1 Prep 4
- ★-★ Ca:Mg:Fe 3:3:2

25 Fig. 8 shows the effect of phosphate binding by aluminium hydroxide, magnesium hydroxide and calcium carbonate over the pH range 3-8. In Fig. 8,

- ▲-▲ Magnesium hydroxide

▼-▼ Calcium carbonate

○-○ Aluminium hydroxide

Fig. 9 shows the individual and mean (\pm 1SEM) urinary phosphate excretion for control rats and those treated with phosphate binding

5 compounds. In particular in Fig. 9, individual values of urinary phosphate excretion ($\mu\text{mol}/24\text{hours}$) were plotted for controls (Δ) and animals treated with $\text{Al}(\text{OH})_3$ (\blacksquare), CaCO_3 (\square), CTFeCa (\bullet), $\text{Mg}(\text{OH})_2$ (\circ), CT100 (\blacklozenge) and CTFeMg (\blacklozenge). Mean (\pm SEM) for each group are presented by points with error bars. * $p < 0.05$ compared to $\text{Al}(\text{OH})_3$ treated animal groups; and

10 Fig. 10 shows the mean (\pm 1SEM) soluble faecal phosphate (g^{-1} dry weight) as a percentage of total (soluble and insoluble) faecal phosphate (g^{-1} dry weight) for control rats and those treated with phosphate binding compounds. In Fig. 10,

* $p < 0.05$ compared to control and CaCO_3 treated animals

15 Δ $p < 0.05$ compared to CaCO_3 treated animals

EXAMPLE 1 - PRELIMINARY INVESTIGATION.

Compounds listed in Table 1 below, known to be effective phosphate binders were selected for investigation. In Table 1, the values indicate respective percentage phosphate binding capacity at each of pH3, pH7
20 and pH8, n indicating the number of trials made for each compound. In the Table, CT100 is a hydrotalcite of the formula $\text{Al}_2\text{Mg}_6\text{OH}_{16}\cdot\text{CO}_3\cdot 4\text{H}_2\text{O}$, commercially available from Crosfield Limited (UK) and CT2000 is the compound CT100 in the form of an undried slurry.

The phosphate binding capacity was measured by mixing 3.2mmol of the
25 compound with 25ml of 20mmol l^{-1} phosphate buffer for 30min at 25°C. For all compounds except CT2000, which compounds were dry powders, the compounds were merely weighed and dosed. For CT2000, the slurry

was dosed in an amount such as to give an equivalent of 1g of a powder dried to constant weight at 40°C. Sodium phosphate and sodium hydrogen phosphate were mixed to provide respective phosphate solutions at pH3, 7 and 8 (HCl being added to provide pH3). The binder
 5 was separated from the solution by centrifugation (5 min, 3000 rpm) and filtration through 0.22µm filters, to provide a supernatant, the phosphate content of which was then measured using a 911 Hitachi autoanalyser with Boehringer Mannheim chemistry. The results are shown in Table 1, in which n refers to the number of observations and the values as the % of
 10 phosphate precipitated out of solution, calculated as follows:

$$100 - [(x/y) \cdot 100]$$

where x = mmol phosphate in solution after precipitation; and
 y = mmol phosphate in solution without precipitation.

Table 1

15	Compound	pH3	pH7	pH8
	Al(OH) ₃ (n=4)	14.7+1.8	6.2+0.4	2.7+1.6
	CaCO ₃ (n=4)	15.3+0.5	9.7+1.8	2.4+1.8
	Mg(OH) ₂ (n=4)	61.1+7.5	45.7+5.9	12.5+3.7
	Ce(OH) ₃ (n=3)	69.8+7.5	57.8+8.9	60.5+1.5
20	CT100 (n=3)	94.6+1.6	91.5+2.5	91.7+0.3
	CT2000 (n=3)	90.7+1.2	87.2+0.0	82.3+1.4

As can be seen from Table 1, each of the hydrotalcite-like materials had a considerably higher phosphate binding capacity over a wider pH range.

25 Dosage relationship curves for the CT compounds and Al(OH)₃ in pH3, 5 and 7 phosphate buffer showed that the CT compounds bound at

least twice as much phosphate as an equivalent weight of $\text{Al}(\text{OH})_3$.

$\text{Al}(\text{OH})_3$ released as much as $20,000\text{--}41,000\mu\text{g l}^{-1}$ of Al^{3+} .

Moreover, although the CT compounds released a considerably lower amount ($17\text{--}66\mu\text{g l}^{-1}$), this would still be likely to provide adverse effects in
5 long time-dosage regimes.

Nevertheless, as indicated by Ookubu (*supra*); it was still thought necessary to include Al^{3+} within the structure of a phosphate binding compound. However, in a test similar to that described above, it was found surprisingly that a compound prepared in a manner similar to that
10 used for preparing CT100 (see Example 3 below) but substituting an equivalent amount of Fe^{3+} gave an excellent phosphate binding capacity, especially at pH3 where a ~70% phosphate binding capacity was achieved, without the risk of release of any aluminium.

EXAMPLE 2 - COMPARISON OF MIXED METAL HYDROXY CARBONATES.

15 Compounds tested:

- (1) a hydroxy carbonate containing a 2:1 ratio Mg: Fe
- (2) a hydroxy carbonate containing a 3:1 ratio of Mg: Fe
- (3) a hydroxy carbonate containing a 3:1 ratio of Ca:Fe
- (4) a hydroxy carbonate containing a 3:3:2 ratio of Ca: Mg: Fe
- 20 (5) CT100, a hydrotalcite of the formula $\text{Al}_2\text{Mg}_6(\text{OH})_{16}\text{CO}_3\cdot 4\text{H}_2\text{O}$, commercially available from Crosfield Limited.
- (6) Altacite, a hydrotalcite of the same formula as CT100, commercially available from Roussell, in the form of an aqueous slurry
- (7) magnesium hydroxide
- 25 (8) aluminium hydroxide

Methods of Measuring Phosphate Binding Capacity

As indicated below, the following methods of measuring phosphate binding capacity were adopted:

Method 1 - 1 gram of each phosphate binder compound (taking hydration of the wet cake compound into account) was added to 25 ml, 40
5 mmol l⁻¹ sodium phosphate buffer adjusted to pH 3, pH 5 or pH 7 as described in Example 3 below. Samples were whirl mixed to ensure homogeneity and gently agitated at room temperature for 30 minutes. Following centrifugation for 5 min at 3000 rpm, the supernatant was filtered through 0.22 µm millipore filters. Soluble phosphate was
10 measured in the supernatant. The percentage phosphate bound by the hydrotalcite was calculated.

Method 2 - As method 1 but using 20 mmol l⁻¹ phosphate buffer.

Method 3 - Milk (250 ml), cornflakes (50 g), bread (2 slices) and marmite (5 g) were mixed in a stomacher for 30 minutes containing 0.01M
15 HCl (so as to simulate the conditions in the stomach). A 20 ml aliquot of food was removed and centrifuged. Phosphate was measured in the supernatant. Two grams of the phosphate binder compound was added to the bulk food slurry and mixed for a further 30 minutes. An aliquot of food was taken and the phosphate measured in the supernatant following
20 centrifugation. Further aliquots were taken after a further 30 and 90 minutes mixing.

In each of the above methods, for each of compounds (1)-(4), where a dry powder was dosed as the phosphate binder, phosphate binding was measured for a given dosage measured after drying to constant weight at
25 40°C. Where a wet cake was dosed (or Altlcite (6) added), an amount equivalent to a given constant dry weight at 40°C was used. For known commercially available binders, a given weight of the material supplied

was used.

Results

Experiment 1: Effect of pH and Ageing on Percentage Phosphate Binding of Mixed Metal Compounds

- 5 Phosphate binding compounds were prepared in the form of a wet slurry. Unaged samples were obtained by filtering and washing the wet slurry to form a wet cake which was tested in this form, while aged samples were obtained by heating the wet slurry to 80°C for two hours prior to filtering of the cake, which was then tested. The percentage phosphate binding of
10 the compounds when used aged or unaged across the pH range 3-7 was investigated in this manner.

Method 1 was used for measuring phosphate binding capacity. The results are shown in Fig. 1.

- The Ca:Fe 3:1 compound (3) bound almost 100% of the phosphate
15 independently of pH. There was no difference between the aged and unaged compound.

- The Mg:Fe compounds (1) and (2) in a 2:1 (prep 1) and 3:1 (prep 2) ratio respectively bound phosphate independently of pH over the range 3-7. The unaged compounds were better phosphate binders than the aged
20 compounds at pH 3-7.

The Ca:Mg:Fe compound (3) also bound phosphate independently of pH; again the unaged was better than the aged compound.

Experiment 2: Effect of pH and Drying on Percentage Phosphate Binding of Mixed Metal Compounds

The percentage phosphate binding of the compounds when used in the dry powder or wet (cake) form across the pH range 3-7 was investigated.

- 5 *Method 1* was used for measuring phosphate binding capacity. The results are shown in Fig. 2.

10 *Unaged* compounds were compared in their wet (cake) form or following drying to constant weight. One gram weight of each compound was used for comparison (hydration of the wet (cake) compound was accounted for e.g. if the hydrotalcite was 20% dry weight (calculated on a constant dry weight at 40°C), 5 grams were used).

15 In all cases, except the Ca:Fe 3:1 compound (3), where there was no difference, the wet (cake) form of the compound was a better phosphate binder than the dry powder form. Whether in the wet or dry form, all of the compounds (1)-(4) bound phosphate independently of pH. Similar results are obtained when using aged compounds in that the wet compound bound more phosphate than the dry powder compound.

Experiment 3: Effect of Increasing Amount of Phosphate Binder Compound on Percentage Phosphate Binding for Various Compounds at pH 3

20

Method 2 was used for measuring phosphate binding capacity. The results are shown in Fig. 3.

At pH 3, $\text{Mg}(\text{OH})_2$, compound (7), was the best phosphate binder. Other studies have however showed this binding is pH dependent, binding almost no phosphate at pH 8. It would therefore have limited use *in vivo*.

5 The compounds Mg:Fe 2:1 (1), Ca:Fe 3:1 (2) and CT100 (5) all bound up to 60-70% of the phosphate. Interestingly, the CT100 bound ~50% more phosphate at any weight than the Altacite (6), despite an identical molecular formulae.

$\text{Al}(\text{OH})_3$, the phosphate binder often used to control serum phosphate levels was relatively ineffective at the range of weights tested.

10 **Experiment 4: Effect of Increasing Amount of Phosphate Binder Compound on Percentage Phosphate Binding for Various Binders at pH 7**

Method 2 was used for measuring phosphate binding capacity. The results are shown in Fig. 4.

15 At pH 7, the Mg:Fe 3:1 compound (2) was the best phosphate binder over the range of weights studied. The CT100 (5) bound at least twice as much phosphate than the Altacite (6) at any weight studied.

Experiment 5: Phosphate Binding in Food

20 *Method 3* was used for measuring phosphate binding capacity. The results are shown in Fig. 5.

The results show that in food, the CT100 (5) was the best phosphate binder, followed by the Fe:Mg 2:1 compound (2). Again, aluminium hydroxide (8) was ineffective. Interestingly, magnesium hydroxide (7), the

best phosphate binder at pH 3, is not the best when used in food. This is probably due to the buffering effect of the food, the initial pH of the slurry being ~ 5. It therefore shows the pH dependency of using magnesium hydroxide as a phosphate binder.

5 Summary

Overall, the results demonstrated:

- The Mg:Fe and Ca:Fe compounds (1)-(4) were efficient phosphate binders across a range of pH's likely to be found in the gastrointestinal tract.
- 10 • Phosphate binding by the MgFe and MgCaFe compounds (1), (2) and (4) but not the CaFe compound (3) was reduced by ageing the compounds.
- Drying the MgFe and MgCaFe compounds (1), (2) and (4) but not the CaFe compound (3) reduced their phosphate binding.
- 15 • The known hydrotalcite compound CT100 (5) bound phosphate in food *in vitro* studies. It also reduced urinary phosphate excretion when given *in vivo* to normal individuals. However, as the new compounds (1)-(4) bound phosphate in water at least as well as CT100 (5) and a number of times better than $\text{Al}(\text{OH})_3$ (8), we would
20 expect they would also bind phosphate *in vivo*. These compounds have the added benefit of not releasing aluminium.
- These new compounds (1)-(4) have a therapeutic potential in the control of serum phosphate levels in patients with end stage renal

failure.

EXAMPLE 3 - FURTHER INVESTIGATIONS OF PHOSPHATE BINDING CAPACITY

Method of Preparation and Measurement

In the following experiments, all chemicals were GPR grade, obtained
5 from BDH. Millipore filters were obtained from Amicon, High Wycombe.

M1. Production of metal co-precipitate preparations

All preparations were synthesised using the following method which, for a
3:1 ratio of $Mg^{2+}:Al^{3+}$ as respective cations $M^{2+}:M^{3+}$, resulted in the
production of the hydrotalcite $Al_2Mg_6(OH)_{16}CO_3 \cdot 4H_2O$.

- 10 Use of calcium or magnesium as the M^{2+} cation and ferric iron as the M^{3+}
cation allowed variations on the above theme to be achieved. By
changing the ratio of the $M^{2+}:M^{3+}$ cations to 1:1, 2:1, 3:1 and 5:1, different
composition materials could be produced. All compounds however had
 CO_3^{2-} as the exchangeable anion.
- 15 For a 3:1 $M^{2+}:M^{3+}$ ratio, salt containing 2 moles of M^{3+} and salt containing 6
moles of M^{2+} were dissolved in 4 litres de-ionised water. In a separate 4
litres, 16 moles NaOH and 5 moles Na_2CO_3 were dissolved. Both
solutions were pumped using peristaltic pumps into a flask with an
overflow at ~2 litres and constantly mixed. The rate of addition of the
20 solutions was such that the mixed solution had a pH of 10.0 - 10.5. After
discarding the first litre, by which time a steady state had been
established, 3-4 litres of overflowing slurry was collected. This was then
vacuum filtered using a Buchner, washed with de-ionised water and re-

filtered leaving a wet 'cake'.

Preparation names and the solution/suspension compositions used for their production are shown in Table 2. Due to the insolubility of calcium sulphate, when used as the M^{2+} salt, constant stirring was necessary to prevent settling.

M2. Production of a metal precipitate mixture

The metals in the solutions/suspensions described in Table 1 were precipitated at the same time by the addition of sodium hydroxide. A preparation was also made by precipitating the calcium and iron separately with sodium hydroxide, the precipitates were then mixed. For this, $Fe_2(SO_4)_3$ (1 mole) and NaOH (6 moles) were mixed in 4 litres de-ionised H_2O . In a separate 4 litres of water, $CaSO_4$ (6 moles), NaOH (12 moles) and Na_2CO_3 (5 moles) were mixed. These two suspensions were then fed with into the flask with an overflow at ~2 litres and constantly mixed.

It proved impossible to alter the rate of addition of the precipitate suspensions such that the mixture had a pH of 10.0 - 10.5. The pH of the mixture fluctuated between ~11.5 and 12.5. After discarding the first litre, 3-4 litres of overflowing slurry was collected. This was then vacuum filtered using a Buchner, washed with de-ionised water and re-filtered leaving a wet 'cake'.

M3. Measurement of metal composition

Preparations were washed and dried to constant dry weight in an oven at ~40°C. One gram was titrated against 1M HCl until a constant pH of 1 was attained. The concentrations of M^{2+} and M^{3+} ions in solution were

measured. For iron and calcium a Hitachi 911 autoanalyser with Boehringer Mannheim chemistry was used, while for magnesium a flame photometric atomic absorption spectroscopy was employed.

NB. Although the methods of analysis adopted here were of high accuracy, the method of sampling was such as to provide only an initial approximate assessment of the actual composition; in the results given below, compare the ratios predicted from the proportions of starting materials (assuming 100% yield) with those of the final preparations measured in this manner.

10 M4. Measurement of phosphate binding

Phosphate binding for the compounds prepared above, when dosed as a dry powder, was measured in each case at a dosage of 1.0 gram dry weight (determined by drying to constant weight at 40°C). Where a wet cake was dosed, an amount equivalent to a 1g dry weight was added.

15 Phosphate binding of the conventional binders, magnesium hydroxide, aluminium hydroxide and calcium carbonate was also measured, in these cases using 1g of material as supplied.

Phosphate binding capacity was determined over a pH range 3-8, approximately the range of pH's found in the normal gastrointestinal tract. 20 40 mmol l⁻¹ sodium phosphate buffers at pH 5, pH 7 and pH 8 were produced by mixing appropriate volumes of 40 mmol l⁻¹ Na₂HPO₄ and 40 mmol l⁻¹ NaH₂PO₄ solutions. A pH 3 phosphate solution was produced by addition of 1 M HCl to a 40 mmol l⁻¹ NaH₂PO₄ solution.

Preparations were suspended in 25 ml 40 mmol l⁻¹ phosphate buffer and 25 whirl mixed to ensure homogeneity. This suspension was then gently agitated at room temperature for 30 minutes followed by centrifugation at

3000 rpm for 5 min. Following filtration of the supernatant through 0.22 μm millipore filters, soluble phosphate was measured using a 911 Hitachi autoanalyser with Boehringer Mannheim chemistry.

5 Phosphate bound was calculated as a percentage of that present in the original solution.

The compositions of solutions used to produce the metal co-precipitate preparations are shown in Table 2 below.

Table 2: Composition of solutions used to produce the metal co-precipitate preparations

	Material name	Moles M^{2+} salt	Moles M^{3+} salt	Moles NaOH	Moles Na_2CO_3
5	Mg:Fe 2:1 (Prep 1)	4 Mole $MgSO_4$	1 Mole $Fe_2(SO_4)_3$	12	5
	Mg:Fe 2:1 (Prep 2)	4 Mole $MgSO_4$	1 Mole $Fe_2(SO_4)_3$	12	5
	Mg:Fe 3:1 (Prep 1)	6 Mole $MgSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5
10	Mg:Fe 3:1 (Prep 2)	6 Mole $MgSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5
	Mg:Fe 3:1 (Prep 3)	6 Mole $MgSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5
15	Mg:Fe 3:1 (Prep 4)	6 Mole $MgSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5
	Ca:Fe 1:1	2 Mole $CaSO_4$	1 Mole $Fe_2(SO_4)_3$	8	5
	Ca:Fe 2:1	4 Mole $CaSO_4$	1 Mole $Fe_2(SO_4)_3$	12	5
	Ca:Fe 3:1 (Prep 1)	6 Mole $CaSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5
20	Ca:Fe 3:1 (Prep 2)	6 Mole $CaSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5
	Ca:Fe 5:1	10 Mole $CaSO_4$	1 Mole $Fe_2(SO_4)_3$	24	5
25	Ca:Fe 3:1 (made with chloride salts)	6 Mole $CaCl_2$	2 Mole $FeCl_2$	16	5
	Ca:Mg:Fe 3:3:2	3 Mole $MgSO_4$ 3 Mole $CaSO_4$	1 Mole $Fe_2(SO_4)_3$	16	5

Results

The following results were obtained.

R1. Predicted and measured metal compositions of the preparations

To determine if the ratio of metal ions in the original solutions was also present in the end preparation, all materials were hydrolysed with 1M HCl and the solution metal ion concentrations measured. The results are shown in Table 3 below. These show that the compounds prepared as above were indeed mixed metal compounds.

Table 3: Predicted and measured metal compositions of the preparations

Material name	Predicted M ²⁺ :M ³⁺ ratio	Measured M ²⁺ :M ³⁺ ratio
Mg:Fe 2:1 (Prep 2)	2:1	1.7:1
Mg:Fe 3:1 (Prep 1)	2:1	2.4:1
Mg:Fe 3:1 (Prep 2)	3:1	2.2:1
Mg:Fe 3:1 (Prep 3)	3:1	2.2:1
Mg:Fe 3:1 (Prep 4)	3:1	2.3:1
Ca:Fe 1:1	1:1	1.3:1
Ca:Fe 2:1	2:1	1.6:1
Ca:Fe 3:1 (Prep 2)	3:1	2.6:1
Ca:Fe 5:1	5:1	1.3:1
Ca:Fe 3:1 (made with Cl- salts)	3:1	1.4:1
Ca:Fe 3:1 (mixing of metals after ppt ⁿ)	3:1	1.1:1
Ca:Mg:Fe	3:3:2	2.9:2.3:2

R2. Phosphate binding

R2.1 Calcium and ferric iron containing preparations

The preparations containing different ratios of calcium to ferric iron were tested for their capacity to bind phosphate.

- 5 The reproducibility of results was demonstrated with reference to a predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 3:1 and this is shown in Table 4 below, while the results obtained for different ratios are shown in Fig. 6 and Table 5 below.

- 10 In the graphs shown in Fig. 6, values plotted are the mean of the two separate experiments.

(i) A predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 3:1

Two different calcium ferric iron preparations with a predicted 3:1 ratio were synthesised. When preparation 2 was hydrolysed, elemental analysis showed the measured calcium to ferric iron ratio to be 2.6:1.

- 15 Insufficient sample of preparation 1 was available for hydrolysis.

Phosphate binding by each preparation was tested in two separate experiments across the pH range 3-8. Binding was reproducible for both preparations at each pH (Table 4). At least 96% of the phosphate present in solution was bound by each preparation at each pH (Fig. 5, Table 4).

Table 4: Reproducibility of phosphate binding for preparations with a predicted 3:1 $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio

	Percentage phosphate binding at			
	pH 3	pH 5	pH 7	pH 8
Prep 1 (exp. 1)	97	98	98	97
Prep 1 (exp. 2)	96	96	97	97
Prep 2 (exp. 1)	98	99	100	100
Prep 2 (exp. 2)	100	99	100	99

(ii) *A predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 1:1*

One calcium ferric iron preparation with a predicted 1:1 ratio was synthesised. Elemental analysis of the hydrolysed material showed the measured calcium to ferric iron ratio to be 1.3:1.

Greater than 50% of the phosphate present in solution was bound by the preparation at pH 3-8 (Fig. 6, Table 5). Phosphate binding was pH dependent. The material bound 28% less phosphate at pH 8 than at pH 3.

(iii) *A predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 2:1*

One calcium ferric iron preparation with a predicted 2:1 ratio was synthesised. Elemental analysis of the hydrolysed material showed the measured calcium to ferric iron ratio to be 1.6:1.

At least 97% of the phosphate present in solution was bound over the pH range 3-8 (Fig. 6, Table 5). There was no pH dependency of the binding.

(iv) *A predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 5:1*

One calcium ferric iron preparation with a predicted 5:1 ratio was
5 synthesised. Elemental analysis of the hydrolysed material showed the measured calcium to ferric iron ratio to be 1.5:1.

At least 95% of the phosphate present in solution was bound over the range pH 3-8 (Fig. 6, Table 5). There was no pH dependency of the binding.

10 (v) *A predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 3:1 made using metal chloride salts*

Due to the insolubility of calcium sulphate, a preparation was made using the soluble salt, calcium chloride. One calcium ferric iron preparation with a predicted 3:1 ratio was synthesised. Elemental analysis of the hydrolysed material showed the measured calcium to ferric iron ratio to be
15 1.4:1.

Greater than 60% of the phosphate present in solution was bound over the pH range 3-8 (Fig. 6, Table 5). Phosphate binding was pH dependent with 31% less precipitated at pH 8 than pH 3.

(vi) *A predicted $\text{Ca}^{2+}:\text{Fe}^{3+}$ ratio of 3:1 made by precipitating the calcium
20 and iron prior to mixing*

A preparation was made to determine whether precipitation of calcium and ferric iron from their sulphates prior to mixing would produce a phosphate

binding material. This compound was prepared as in methods M2. The predicted ratio of calcium to ferric iron was 3:1 although, the ratio measured following acid hydrolysis was 1.1:1.

Greater than 75% of the phosphate present in solution was bound over the pH range 3-8 (Fig. 6, Table 5). The binding was pH dependent to a small degree, at pH 8, 8% less phosphate was bound than at pH 3.

Table 5: Phosphate binding by the calcium ferric containing preparations at pH 3-8

Predicted Ca ²⁺ Fe ³⁺ ratio	Percentage phosphate bound			
	pH 3	pH 5	pH 7	pH 8
1:1	75	72	63	54
2:1	99	95	99	98
3:1*	98	99	100	100
5:1	97	96	99	98
3:1 (made with chloride salts)	92	87	72	64
3:1 (with prior ppt ⁿ of metals)	85	84	80	78

*Preparation 2 (exp. 1) of Table 4 also included for comparison

R2.2 Preparations containing magnesium and ferric iron

A number of preparations containing different ratios of magnesium to ferric iron were tested for their ability to bind phosphate.

5 The reproducibility of results was demonstrated in each case and these results are shown in Tables 6-8 below, while a comparison of the results is shown in Fig. 7.

(i) A predicted $Mg^{2+}:Fe^{3+}$ ratio of 3:1

Four magnesium ferric iron preparations were synthesised with the predicted ratio of 3:1. Preparation 1 had an actual $Mg^{2+}:Fe^{3+}$ ratio of 2.4:1
10 Preparations 2, 3 and 4 had measured $Mg^{2+}:Fe^{3+}$ ratios of 2.2:1, 2.2:1 and 2.3:1 respectively.

Preparation 1 bound at least 60% of the phosphate over the pH range 3-7. Preparations 2, 3 and 4 bound at least 40%, 50% and 30% of the phosphate respectively over the pH range 3-8 (Fig. 7, Table 6):
15 Phosphate binding by preparation 4 was reproducible (Table 6). A shortage of material meant binding experiments on preparations 1, 2 and 3 were carried out once.

The three preparations studied over the pH range 3-8 all displayed pH dependency in their phosphate binding. Preparations 2 and 3 bound 44%
20 and 29% less phosphate respectively at pH 8 than pH 3. Preparation 4 bound a mean of 21% less phosphate at pH 8 than pH 3.

Table 6: Phosphate binding for preparations with the predicted 3:1
 $Mg^{2+}:Fe^{3+}$ ratio

	Percentage phosphate binding at			
	pH 3	pH 5	pH 7	pH 8
Prep 1	60	58	61	-
Prep 2	79	76	55	44
Prep 3	75	73	63	53
Prep 4 (exp. 1)	41	40	34	37
Prep 4 (exp. 2)	45	39	36	32

(i) A predicted $Mg^{2+}:Fe^{3+}$ ratio of 2:1

Two magnesium ferric iron preparations with a predicted 2:1 ratio were synthesised. Elemental analysis of preparation 2 following hydrolysis showed the measured magnesium to ferric iron ratio to be 1.7:1.

Insufficient sample was available to study the elemental composition of preparation 1.

Preparation 1 bound greater than 60% of the phosphate across the pH range 3-7. Preparation 2 reproducibly bound greater than 30% of the phosphate across the pH range 3-8 (Table 7, Fig. 7). This was pH dependent with a mean of 27% less phosphate being bound at pH 8 than pH 3.

Table 7: Phosphate binding for preparations with the predicted 2:1Mg²⁺:Fe³⁺ ratio

	percentage phosphate binding at			
	pH 3	pH 5	pH 7	pH 8
Prep 1	77	75	65	-
Prep 2 (exp. 1)	50	48	41	37
Prep 2 (exp. 2)	42	39	38	30

2.3 A magnesium, calcium and ferric iron containing preparation(i) *A predicted Ca²⁺:Mg²⁺:Fe³⁺ ratio of 3:3:2*

One calcium magnesium ferric iron preparation with a predicted 3:3:2 ratio was synthesised. When this was hydrolysed, elemental analysis showed the measured calcium to magnesium to ferric iron ratio to be 2.9:2.3:2.

This compound bound greater than 45% of the phosphate in solution across the pH range 3-8 (Fig. 7). Two separate experiments showed that the phosphate binding was reproducible (Table 8). Binding was pH dependent with a mean of 36% less phosphate precipitated at pH 8 than pH 3.

Table 8: Phosphate binding for preparation with a predicted 3:3:2Ca²⁺:Mg²⁺:Fe³⁺ ratio

	Percentage phosphate binding at			
	pH 3	pH 5	pH 7	pH 8
exp. 1	80	77	65	54
exp. 2	80	78	64	48

R2.4 Phosphate binding by conventional compounds

The compounds aluminium hydroxide, magnesium hydroxide and calcium carbonate were also tested for their ability to bind phosphate. The method was as previously described in M4.

- 5 All compounds were tested twice and showed reproducible phosphate binding across the pH range studied and the results are shown in Fig. 8 and Table 9 below. In Fig. 8, values plotted are the mean of two separate experiments for each compound.

10 As can be seen, phosphate binding was pH dependent with a mean 2.4 fold increase in binding by $\text{Al}(\text{OH})_3$ at pH 3 compared to pH 8. $\text{Mg}(\text{OH})_2$ bound a mean 3.7 times more phosphate at pH 3 than pH 8. CaCO_3 bound a mean of 5.9 times more phosphate at pH 3 than pH 8.

Table 9: Phosphate binding by $\text{Al}(\text{OH})_3$, $\text{Mg}(\text{OH})_2$ and CaCO_3

	Percentage phosphate binding at			
	pH 3	pH 5	pH 7	pH 8
$\text{Al}(\text{OH})_3$	20	19	18	9
$\text{Al}(\text{OH})_3$	30	25	23	12
$\text{Mg}(\text{OH})_2$	81	82	54	17
$\text{Mg}(\text{OH})_2$	87	80	58	28
CaCO_3	69	63	30	8
CaCO_3	72	70	43	16

EXAMPLE 4 - CALCIUM SULPHATE AS PHOSPHATE BINDER

The following compounds were tested as phosphate binders:

1. Anhydrous calcium sulphate treated with sodium hydroxide
2. Anhydrous calcium sulphate
- 5 3. $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$.
4. Ferrous/ferric co-precipitate
5. Ferric precipitate

1. *Anhydrous calcium sulphate treated with sodium hydroxide*

This was prepared by mixing anhydrous calcium sulphate (CaSO_4) (0.1
10 moles), with sodium hydroxide (NaOH) (0.2 moles) in 100 ml de-ionised
water for 30 minutes at room temperature. The mixture was centrifuged
for 2 min at 3000 rpm and the supernatant discarded. The residue was
washed by mixing with 100 ml water for 5 minutes followed by
centrifugation for 2 min at 3000 rpm. The supernatant was discarded and
15 the washing procedure repeated a further three times. The resultant solid
was heated to constant dry weight at 60°C.

2. *Anhydrous calcium sulphate*

A commercially available dry anhydrous calcium sulphate powder was
used.

20 3. *Calcium sulphate dihydrate*

A commercially available calcium sulphate dihydrate powder was used.

4. *Ferrous/ferric co-precipitate*

This was prepared by co-precipitating ferrous sulphate FeSO_4 and ferric sulphate $\text{Fe}_2(\text{SO}_4)_3$ with sodium hydroxide to obtain a hydrated iron oxide compound. The predicted $\text{Fe}^{2+}:\text{Fe}^{3+}$ ratio was 3:1.

5 5. *Ferric Precipitate*

This was prepared by mixing ferric sulphate ($\text{Fe}_2(\text{SO}_4)_3$)(0.1 moles), with sodium hydroxide (NaOH)(0.3 moles) in 100 ml de-ionised water for 30 minutes at room temperature.

10 The mixture was centrifuged for 5 min at 3000 rpm and the supernatant discarded.

The precipitate was washed by mixing with 100ml water for 5 minutes followed by centrifugation for 5 min at 3000 rpm. The supernatant was discarded and the washing procedure repeated a further 3 times.

The precipitate was heated to constant dry weight at 60°C.

15 **Phosphate binding**

The phosphate binding capacity of each of the above materials was measured as described above in Example 3, using one gram of each compound in 25 ml phosphate solution 40 mmol l^{-1} , pH 3-8.

The results are shown in Table 10 below.

Table 10: Phosphate binding over the pH range 3-8 by alkali treated calcium sulphate, anhydrous and hydrated calcium sulphates and an $\text{Fe}^{2+}:\text{Fe}^{3+}$ compound with a predicted 3:1 ratio and an Fe^{3+} compound

Compound	Percentage phosphate bound at			
	pH 3	pH 5	pH 7	pH 8
Treated CaSO_4	100	100	100	100
Anhydrous CaSO_4	2	7	47	55
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0	0	57	89
$\text{Fe}^{2+}:\text{Fe}^{3+}$ 3:1	26	18	33	-
Fe^{3+}	56	59	56	41

From the above, it can be seen firstly that mixed metal compounds preferably containing each of a ferric cations and at least one of magnesium, calcium, lanthanum and cerium cation, and at least one of hydroxyl and carbonate anions and optionally at least one of sulphate, chloride and oxide have excellent phosphate binding capacity at a buffer pH relevant to physiological conditions in the gastrointestinal tract.

In particular, they show excellent phosphate binding capacity over a pH range of from 2-8, especially 3-7, and are therefore able to bind phosphate both in the stomach region (upper tract) where the pH would normally be about 3-4, up to 7, possibly depending upon the pH of the binder itself, and also in the lower tract, for example in the duodenum or jejunum, where the pH is likely to be ≥ 7 .

In view of this high binding capacity, lower dosages are possible.

Moreover, for the same weight of phosphate binding compound a mixed calcium/ferric compound contains less ferric ion than the corresponding compound containing iron alone. This allows a small *in vivo* dosage of iron for at least the same phosphate binding capacity, thus

raising the likely tolerance of a patient to the dosage given.

The phosphate binding capacity of the mixed magnesium/ferric compound, is also remarkably less pH dependent as compared with magnesium hydroxide. Moreover, the magnesium tends to be stabilised,
5 leading to a lower expected release thereof when administered *in vivo* with expected reduced side effects such as hypermagnesaemia. Likewise, the iron tends to be stabilised, leading to a lower expected release thereof *in vivo*, with an expected reduction in the free radical formation *in vivo* often encountered with Fe^{3+} ions, so leading to less
10 damage of membrane tissue.

It is also found, particularly surprisingly, that the above also applies to calcium sulphate after treatment thereof with an alkali solution.

EXAMPLE 5 - MIXED METAL HYDROXY CARBONATE AS PHOSPHATE BINDERS - IN VIVO STUDY IN RATS

15 MATERIALS AND METHODS

The following chemicals unless otherwise stated were GPR grade from BDH/Merck (Poole, UK): CaSO_4 , $\text{Fe}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$ (technical grade), MgSO_4 , CaCO_3 , NaOH , 70% Nitric acid (redistilled, 99.99% purity). $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ were obtained from Sigma (Poole UK). CT100 was
20 obtained from Crosfield Ltd (Warrington, UK).

Phosphate binders were incorporated into the standard rat diet rat/mouse maintenance No1 food obtained from Lilico (Betchworth, Surrey UK).

Production of the CT compounds

CTFeCa and CTFeMg were mixed metal hydroxycarbonates, having a predicted ratio of Mg^{2+} or Ca^{2+} : Fe^{3+} of 3:1, produced in the laboratory following a standard laboratory procedure for mixed metal hydroxy carbonate preparations as described in Example 3 (M2). This metal²⁺ sulphate, 6 moles, and metal³⁺ sulphate, 2 moles, were dissolved in 4 litres de-ionised H_2O . In a separate flask, 16 moles NaOH and 5 moles Na_2CO_3 were dissolved in 4 litres de-ionised H_2O . The two solutions were pumped using peristaltic pumps into a flask with an overflow at ~2 litres, the rate of addition of the solutions was such that when mixed, the resulting suspension had a pH of 10.0 - 10.5. After discarding the first litre, by which time a steady state had been established, 3-4 litres of overflowing slurry was collected. This was vacuum filtered using a Buchner flask and washed with 1 litre de-ionised water three times. To allow incorporation into rat food, the wet "cake" compound was dried to constant dry weight at 50°C and ground with a mortar and pestle.

In vivo studies in the rat

Twenty eight rats (Sprague-Dawley strain), weight range 275-307 grams were divided into seven groups, each consisting of four animals (in Tables 11-14, n =4). The phosphate binders were incorporated into the rat food at a concentration of 1% (w/w). Each group of rats was fed a single diet *ad libitum* for seven days and had unlimited access to de-ionised water. Animals were then weighed and transferred to metabowls for 24 hours where they received 18 grams of the control diet and unlimited access to water. Total 24 hour urine and faecal output was collected during this time. At the end of the treatment periods, animals were reweighed and a blood sample was obtained via the carotid artery following anaesthetisation with sodium

pentobarbitone (Sagatal) 0.1ml/100g body weight of a 60mg/ml solution.

Preparation of faeces and urine

Due to the design of the metabowls, the rat faeces were unavoidably contaminated with control food from the diet and there was also slight contamination of the urine. Prior to analysis, food was therefore separated from the urine by 5 minutes centrifugation at 1500 rpm. The food pellet was discarded. Contaminating particulate food was removed from the faeces using forceps and the stool sample weighed.

Total faecal samples from each animal were mixed to ensure a homogeneity and duplicate one gram aliquots weighed. The percentage hydration of the stool was calculated following freeze drying to constant weight.

For measurement of total faecal phosphate and metal ion content, freeze dried faeces was ground with a mortar and pestle and 200mg hydrolysed by heating to 70°C for 4 hours with 7ml concentrated nitric acid in polypropylene test tubes. The faecal digests were diluted to 50ml with de-ionised water in acid washed 125ml Nalgene containers.

For measurement of soluble faecal phosphate and metal ion content, a 1.5 gram aliquot of stool was suspended in 15 ml deionised water. Following homogenisation and centrifugation at 3000 rpm for 45 minutes, the supernatant was filtered through glass wool to remove contaminating particulate matter and stored at -20°C.

Analytical methods

Phosphate, iron and calcium were determined in the faecal digest solutions,

urine and serum using standard Boehringer Mannheim chemistry on a Hitachi 911 autoanalyser. Magnesium was measured in the faecal digest solutions, urine and serum using flame photometry atomic absorption spectrometry. Urine and serum aluminium were measured using graphite
5 furnace atomic absorption spectrometry.

Differences between treatment groups was assessed using Students t-test with $p < 0.05$ being considered significant.

Procedure

All animals were weighed daily during the course of the study to ensure that
10 food modified by the addition of phosphate binding compounds did not affect weight gain. During the seven day equilibration period, groups of animals treated with CTFeCa, CTFeMg, $Mg(OH)_2$, $CaCO_3$ or CT100 showed a range of mean weight gains from 38 - 53 grams. Rats treated with $Al(OH)_3$ showed a mean weight gain of 3 grams. The control group demonstrated a
15 reluctance to eat the standard RMI diet (without addition of phosphate binders). After four days, it was necessary to switch them to a control diet (Lilico). These control animals showed a mean weight loss of 17.5 grams during this seven day period. Soluble phosphate was measured in the Lilico diet and found to be $6.8 \mu\text{mol g}^{-1}$, similar to that of the RMI diet without
20 addition of binders, $7.5 \mu\text{mol g}^{-1}$.

Following feeding with the modified diets for 7 days, animals were transferred to metabowls for collection of total 24 hour faecal and urine excretion. To ensure that any contamination of faeces and urine by food was similar for the different groups, each animal was given a restricted 18 grams of control diet
25 (Lilico). During this period, control animals gained a mean of 3 grams in weight. Other animal groups showed a mean weight loss of 2 -22 grams.

Results

Measurement of Urine and Faecal Phosphate Excretion.

Reduced phosphate absorption achieved when a dosage of the inorganic compound is ingested with food is manifested by a low urine phosphate content, a high total faecal phosphate content and a low ratio of soluble faecal phosphate content: total faecal phosphate content (Table 11).

Differences in urinary phosphate concentration between animals groups could be explained by significant differences in urine volume. Renal phosphate excretion was therefore expressed as total (μmol) per 24 hours. Animals treated with $\text{Al}(\text{OH})_3$ and CaCO_3 excreted 1259 ± 279 μmol phosphate and 857 ± 25 μmol phosphate (mean \pm SEM) respectively (Figure 9, Table 11). These values were significantly higher than from rats treated with CTFeCa, CTMgFe, CT100 or $\text{Mg}(\text{OH})_2$ mean 71 ± 44 μmol , 13 ± 4 μmol , 26 ± 11 μmol and 65 ± 53 μmol phosphate respectively. No group treated with phosphate binding compounds showed a significant difference in urinary phosphate excretion compared to the controls, mean 466 ± 188 μmol . This may be explained by a lower food intake by the control animals, demonstrated by their mean weight loss over the course of the study.

To indicate whether phosphate binders were precipitating phosphate in the rat gastrointestinal tract, total stool phosphate (bound and soluble) and soluble stool phosphate (unbound) were measured. To control for variations in faecal output and faecal hydration between groups, faecal phosphate was expressed as μmol phosphate g^{-1} dry weight faeces. Total (soluble and insoluble) phosphate g^{-1} dry weight faeces did not differ significantly between any of the treatment groups. Faeces from animals treated with CTFeCa

contained significantly less soluble phosphate than the controls or the animals treated with CaCO_3 (Table 11). Mean soluble phosphate g^{-1} dry weight faeces as a percentage of mean total phosphate g^{-1} dry weight faeces was 41.9%, 44.8%, 55.9%, 60.7% and 45.0% for animals treated with

5 CTF_{Fe}Ca, $\text{Mg}(\text{OH})_2$, $\text{Al}(\text{OH})_3$, CT100 and CTF_{Fe}Mg respectively. Soluble phosphate consisted of 79.0% of the total in the control group and 85.5% of the total in the CaCO_3 treated group (Figure 10). These results demonstrate the effectiveness of the CT compounds as binders, decreasing the available phosphate compared to controls and CaCO_3 treated animals.

Table 11

Mean (\pm 1SEM) urine and faecal phosphate excretion for control rats and those treated with phosphate binding compounds.

		Control	Al(OH) ₃	CaCO ₃	CTFeCa
5	Urine phosphate μmol (n=4)	466 \pm 188*	1259 \pm 279	857 \pm 25	72 \pm 44*
	Total faecal phosphate $\mu\text{mol g}^{-1}$ dry weight faeces (n=4)	150 \pm 32	188 \pm 26	213 \pm 16	181 \pm 12
10	Soluble faecal phosphate $\mu\text{mol g}^{-1}$ dry weight faeces (n=4)	120 \pm 6	96 \pm 9	181 \pm 9 Δ	73 \pm 12 ϕ
		Mg(OH) ₂	CT100	CTFeMg	
	Urine phosphate μmol (n=4)	65 \pm 53*	26 \pm 11*	13 \pm 4*	
15	Total faecal phosphate $\mu\text{mol g}^{-1}$ dry weight faeces (n=4)	183 \pm 17	181 \pm 40	206 \pm 34	
	Soluble faecal phosphate $\mu\text{mol g}^{-1}$ dry weight faeces (n=4)	87 \pm 14	100 \pm 15	128 \pm 8	

20 * p<0.05 compared to Al(OH)₃ and CaCO₃ treated animals.

Δ p<0.05 compared to all groups

ϕ p<0.05 compared to Control and CTFeMg treated animals.

Measurement of Metal Extraction and Retention

Urine aluminium excretion, serum aluminium concentration

- Urine and serum aluminium concentrations were measured using graphite furnace atomic absorption spectroscopy. For the animals taking $\text{Al}(\text{OH})_3$ or CT100, mean serum aluminium concentrations were not significantly higher than serum aluminium from control animals (Table 12). Surprisingly, animals treated with CTFeCa and CTFeMg showed the highest mean serum aluminium concentrations, both significantly higher than animals treated with $\text{Mg}(\text{OH})_2$, $\text{Al}(\text{OH})_3$, CaCO_3 or controls.
- Due to significant differences in total urine volume between different animal groups, aluminium was expressed as μg excreted. For animals treated with $\text{Al}(\text{OH})_3$, mean urinary Al^{3+} excretion was at least 2 fold higher than animals treated with any other phosphate binder (Table 12). The animals treated without binders (control diet) surprisingly excreted more aluminium than the animals treated with $\text{Al}(\text{OH})_3$.

Measurement of urine calcium excretion, serum calcium concentration

- Total urinary calcium excretion from CaCO_3 treated animals was not significantly different to controls or animals treated with CTFeCa or $\text{Al}(\text{OH})_3$. CaCO_3 treated animals excreted significantly more calcium than animals treated with $\text{Mg}(\text{OH})_2$, CT100 or CTFeMg (Table 13).

Control animals and those treated with $\text{Al}(\text{OH})_3$ had significantly higher serum calcium concentrations than animals supplied with any other treatment (Table 13). Rats treated with CaCO_3 had significantly higher serum calcium than those treated with $\text{Mg}(\text{OH})_2$, CT100 or CTFeCa.

Measurement of urine magnesium excretion

Urinary magnesium excretion following treatment with the compounds CT100 and CTFeMg was higher although not significantly so compared to the control animals (Table 14). Following $\text{Mg}(\text{OH})_2$ administration, urine
5 magnesium excretion was significantly higher than the control group or animals treated with any other binder.

Measurement of urinary and serum iron concentration

In all urine samples from all treatment groups, iron concentration was at the limit of detection of the method employed ($>1\mu\text{mol l}^{-1}$).

- 10 Release of iron from the phosphate binders was of concern and so serum iron concentrations were measured in all animals. There was however no significant difference in serum iron concentration between any of the treatment groups (Table 14).

Table 12:

Mean (± 1 SEM) urine aluminium excretion, mean (± 1 SEM) serum aluminium concentration for control rats and those treated with phosphate binding compounds.

Treatment	Urine aluminium μg (all n=4)	Serum aluminium $\mu\text{mol l}^{-1}$
Control	$1.23 \pm 0.05\alpha$	0.45 ± 0.04
$\text{Al}(\text{OH})_3$	$1.07 \pm 0.38\beta$	0.38 ± 0.03
CaCO_3	0.50 ± 0.21	0.33 ± 0.05
CTFeCa	0.18 ± 0.12	$0.66 \pm 0.07^*$
$\text{Mg}(\text{OH})_2$	0.17 ± 0.07	0.35 ± 0.08
CT100	0.26 ± 0.09	0.65 ± 0.24
CTFeMg	0.31 ± 0.09	$0.65 \pm 0.05^*$

* $p < 0.05$ compared to $\text{Mg}(\text{OH})_2$, $\text{Al}(\text{OH})_3$, CaCO_3 and control treated animals

α $p < 0.05$ compared to $\text{Mg}(\text{OH})_2$, $\text{Al}(\text{OH})_3$, CaCO_3 , CTFeMg, CT100 and CTFeCa treated animals

β $p < 0.05$ compared to $\text{Mg}(\text{OH})_2$, $\text{Al}(\text{OH})_3$, CTFeMg, CT100 and CTFeCa treated animals

Table 13:

Mean (\pm 1SEM) urine calcium excretion, mean (\pm 1SEM) serum calcium concentration for control rats and those treated with phosphate binding compounds.

Treatment	Urine calcium μ mol	Serum calcium mmol l ⁻¹
Control	317 \pm 94	3.29 \pm 0.16 (n=3) α
Al(OH) ₃	539 \pm 242	3.27 \pm 0.07 (n=3) α
CaCO ₃	472 \pm 17*	2.93 \pm 0.09 (n=4) β
CTFeCa	333 \pm 80	2.48 \pm 0.10 (n=4)
Mg(OH) ₂	360 \pm 62	2.58 \pm 0.05 (n=3)
CT100	314 \pm 20	2.54 \pm 0.07 (n=4)
CTFeMg	300 \pm 34	2.69 \pm 0.07 (n=4)

*p >0.05 compared to CT100, Mg(OH)₂ and CTFeMg treated animals

α p>0.05 compared to CTFeCa, Mg(OH)₂, CT100 and CTFeMg treated animals

β p<0.05 compared to Mg(OH)₂, CT100 or CTFeCa treated animals

Table 14:

Mean (\pm 1SEM) urine magnesium excretion, mean (\pm 1SEM) serum iron concentration for control rats and those treated with phosphate binding compounds.

Treatment	Urine magnesium μ mol (all n=4)	Serum iron mmol l ⁻¹
Control	6.3 \pm 1.8	37.8 \pm 11.2 (n=3)
Al(OH) ₃	9.7 \pm 0.6	38.5 \pm 15.9 (n=3)
CaCO ₃	8.7 \pm 1.8	41.9 \pm 10.8 (n=4)
CTFeCa	5.9 \pm 1.2	23.9 \pm 5.1 (n=4)
Mg(OH) ₂	17.3 \pm 2.3*	29.4 \pm 7.9 (n=3)
CT100	9.2 \pm 0.6	39.5 \pm 10.8 (n=4)
CTFeMg	11.4 \pm 0.7	48.5 \pm 12.5 (n=3)

* p<0.05 compared to all groups

Discussion of Results

As phosphate binders are administered in relatively large doses over long periods of time, metal ion release, absorption and toxicity is of prime concern. Serum aluminium concentration in $\text{Al}(\text{OH})_3$ or CT100 treated animals was not significantly higher than animals treated with any other binder. This is in agreement with a human study which reported no increase in serum aluminum, measured up to seven hours after administration of 6 grams hydrotalcite (CT100) [Van der Voet and de Wolff, Clin. Tox. (1986-87), 24, 545-553]. As only ~0.1% of an ingested aluminum dose is absorbed [Powell and Thompson, Proc. Nutr. Soc., (1993) 52, 241 - 253], changes in the large serum volume are at the limits of accurate measurement.

We therefore measured urinary aluminium excretion as an indicator of intestinal uptake. Animals treated with $\text{Al}(\text{OH})_3$ excreted at least 2 fold more aluminium than those treated with any other binder and four fold more than CT100 treated rats. Conclusions as to the relative benefits of CT100 in terms of aluminium release are however limited due to the high urinary excretion from the controls.

Release and absorption of iron from the CTFeCa and CTFeMg binders was of concern as body iron content is regulated by absorption from the gastrointestinal tract [McCance and Widdowson, Lancet, (1937) 2, 680-684]. There is no physiological route by which it can be excreted and daily losses are low, urine <0.1 mg, skin losses 0.2-0.3 mg and faeces 0.6 mg [Bothwell, Nutr. Ron. (1995), 53, 237-245]. Animals treated with CTFeCa or CTFeMg did not show an increase in serum iron compared to animals treated with non iron containing binders or controls and as expected, urine iron excretion was at the limit of detection in all groups.

- Compared to animals treated with any other binder, there was at least a 66% and 113% increase in soluble faecal iron in CTFeCa or CTFeMg treated animals respectively. Whether this was absorbable was beyond the scope of this study as complex factors including diet and iron store size influence non-haem iron uptake [Bothwell, Supra: Cook, Am. J. Clin. Nutr. (1990), 51, 301-308]. However, as a number of haemodialysis patients are anaemic, an increased iron load may be beneficial [Remussi and Rossi, in The Kidney (Ed. Brenner, BM), W. B. Saunders, Philadelphia, (1996), Chapter 50, pp 2170 - 2186].
- 10 Different magnesium salts have been shown to have efficacy as phosphate binders. Magnesium carbonate has been shown to be an efficient binder [O'Donovan et.al., Lancet, (1986), 51, 880-881] while magnesium hydroxide has been shown to be ineffective or poorly tolerated [Guillot et al., Nephron, (1982), 30, 114-117; Oe et al., Clin. Nephrol, (1987), 28, 180-185]. Care must be taken though to avoid over administration due to the laxative effects of magnesium. In this study none of the animal groups treated with Mg(OH)₂, CT100 or CTFeMg showed an increase in faecal hydration compared to the controls suggesting a dose that was well tolerated by the animals. Neither urine nor serum magnesium were elevated in CTFeMg or CT100 treated animals, suggesting that Mg absorption from these compounds was low.
- 15
20

In summary, CT100, CTFeMg and CTFeCa are all high capacity phosphate binders when administered *in vivo* to rats at low doses. This study indicates they are likely to have limited toxicity although long time course studies are required to evaluate iron, aluminium and magnesium absorption. These compounds may present effective alternatives to the currently prescribed phosphate binders.

25

CLAIMS

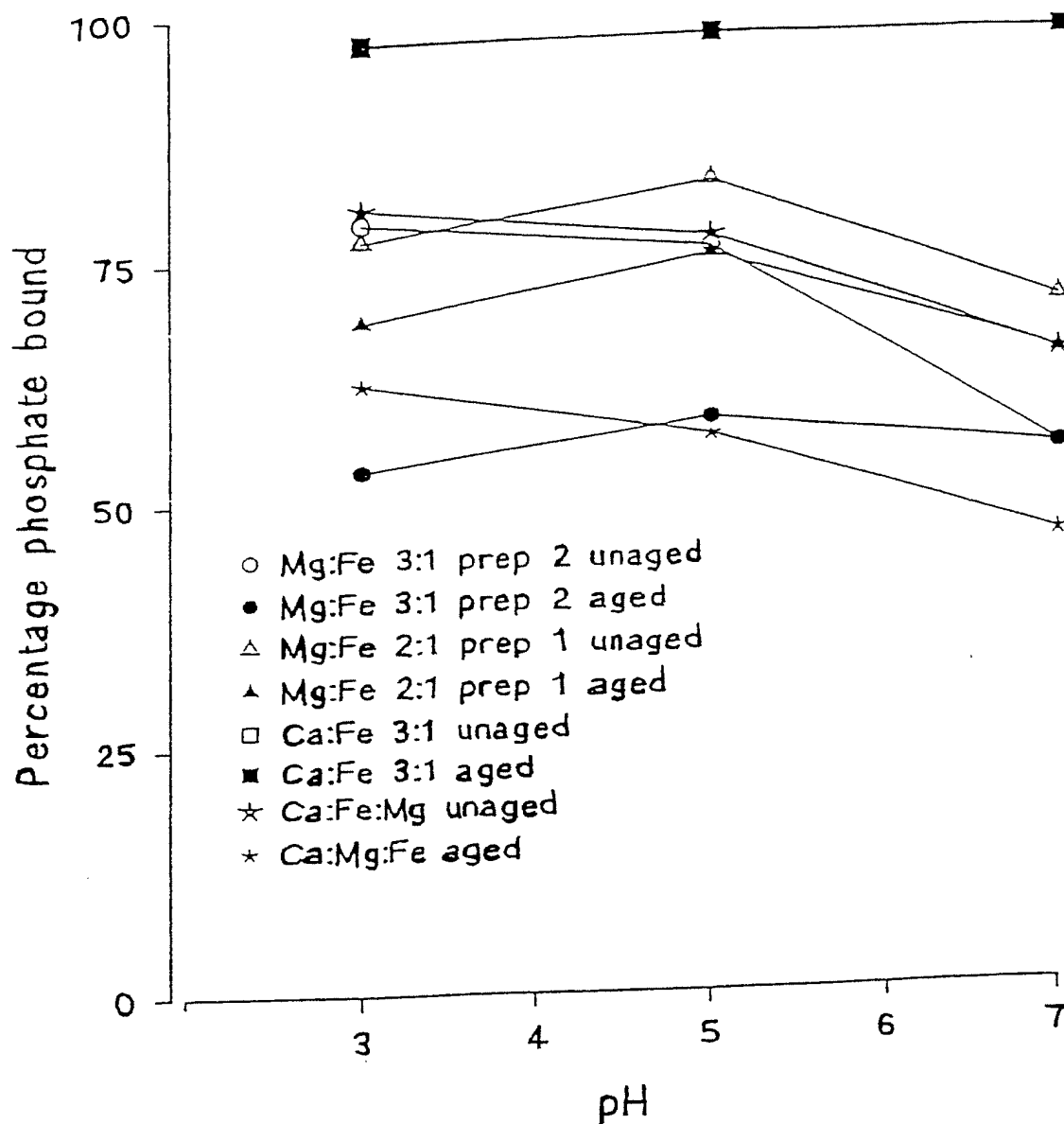
1. A mixed metal compound for pharmaceutical use which is free from aluminium and has a phosphate binding capacity of at least 30%, by weight of the total weight of phosphate present, over a pH range of from 2-8.
- 5 2. A mixed metal compound according to claim 1 having a phosphate binding capacity of at least 30%, by weight of the total weight of phosphate present, over a pH range of from 3-7.
3. A mixed metal compound according to claim 1 or claim 2, which is a co-precipitate.
- 10 4. A mixed metal compound according to any preceding claim, wherein the metals are iron(III) and at least one of magnesium, calcium, lanthanum and cerium.
5. A mixed metal compound according to any preceding claim, which contains at least one of hydroxyl and carbonate anions.
- 15 6. A mixed metal compound according to claim 5, which additionally contains at least one of sulphate, chloride and oxide.
7. Use, in a method of preparing a medicament for treatment of hyperphosphataemia, of a mixed metal compound according to any preceding claim.
- 20 8. Use, in a method of preparing a medicament for treatment of hyperphosphataemia, of a metal sulphate material selected from at least one of calcium, lanthanum and cerium sulphate compounds treated with an alkali solution.
9. Use according to claim 8, wherein the alkali is sodium hydroxide.
- 25 10. Use according to claim 9, wherein the metal sulphate is treated with an aqueous sodium hydroxide solution.
11. Use according to any one of claims 8 to 10, wherein the metal sulphate compound is calcium sulphate.

12. A metal sulphate material for pharmaceutical use selected from at least one of calcium, lanthanum and cerium sulphate compounds treated with an aqueous solution of an alkaline hydroxide, which said material comprises a solid material.
- 5 13. A metal sulphate material for pharmaceutical use, which metal sulphate material comprises a solid material selected from at least one of calcium, lanthanum and cerium sulphate compounds and has a phosphate binding capacity of at least 30%, by weight of the total weight of phosphate present, over a pH range of from 2-8.
- 10 14. A method of preparing a metal sulphate material, which method comprises treating a metal sulphate comprising a solid material selected from at least one of calcium, lanthanum and cerium sulphate with an alkali solution.
- 15 15. A method according to claim 13, wherein the metal sulphate is calcium sulphate.

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Figure 1:

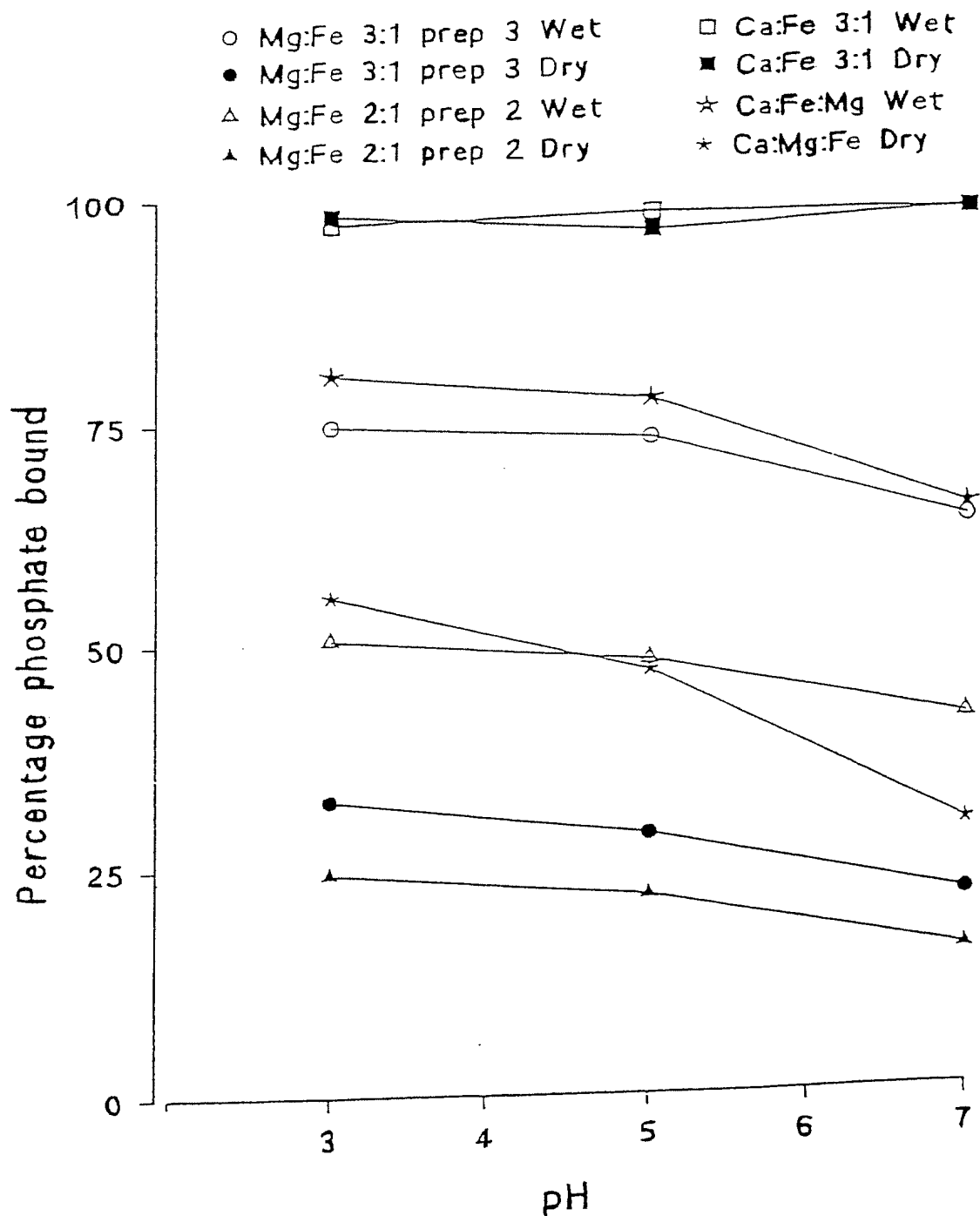
Effect of pH and ageing on percentage phosphate binding of mixed metal compounds



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Figure 2:

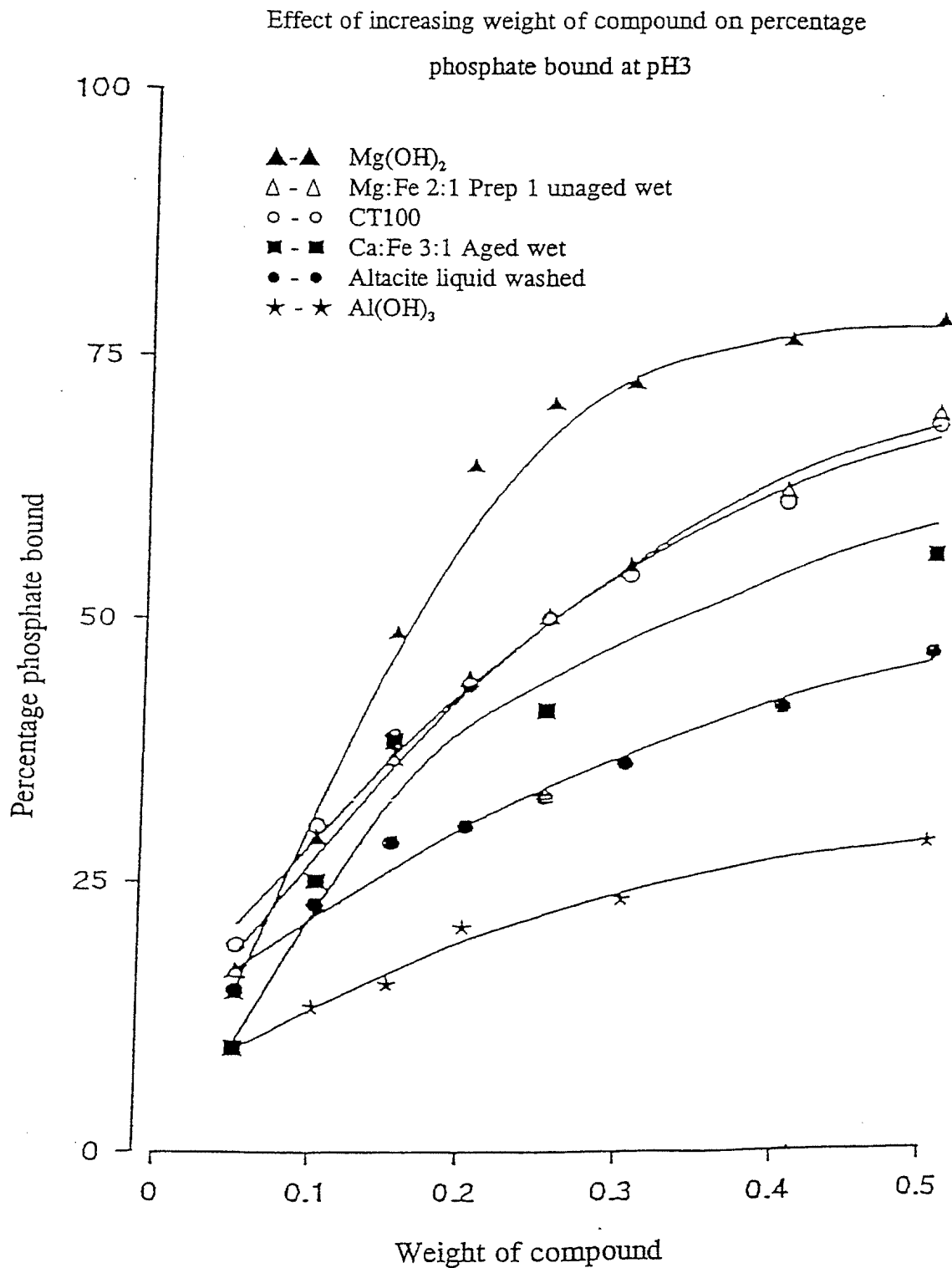
Effect of pH and drying on percentage phosphate binding of mixed metal compounds



SUBSTITUTE SHEET (RULE 26)

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Figure 3

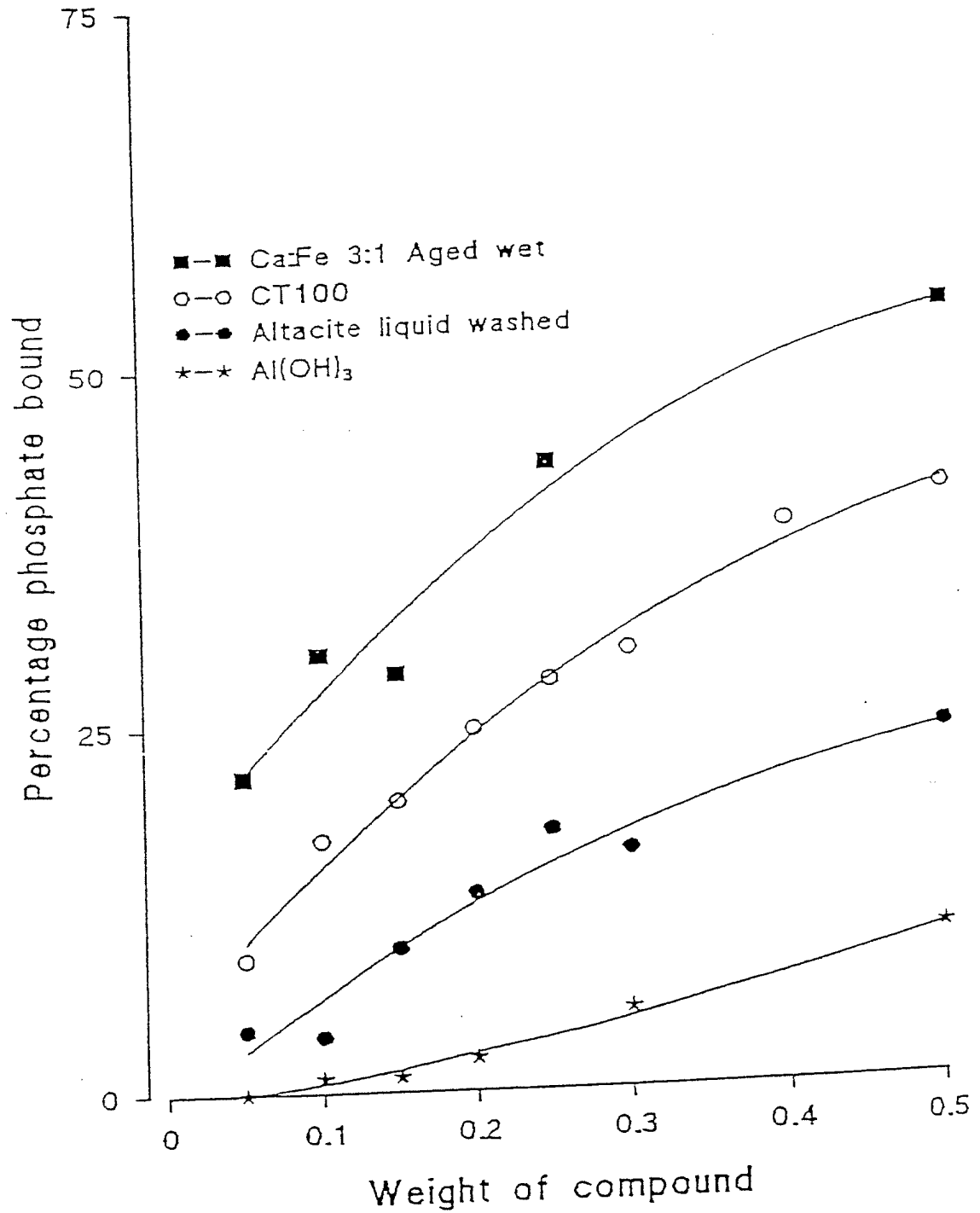


SUBSTITUTE SHEET (RULE 26)

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Figure 4

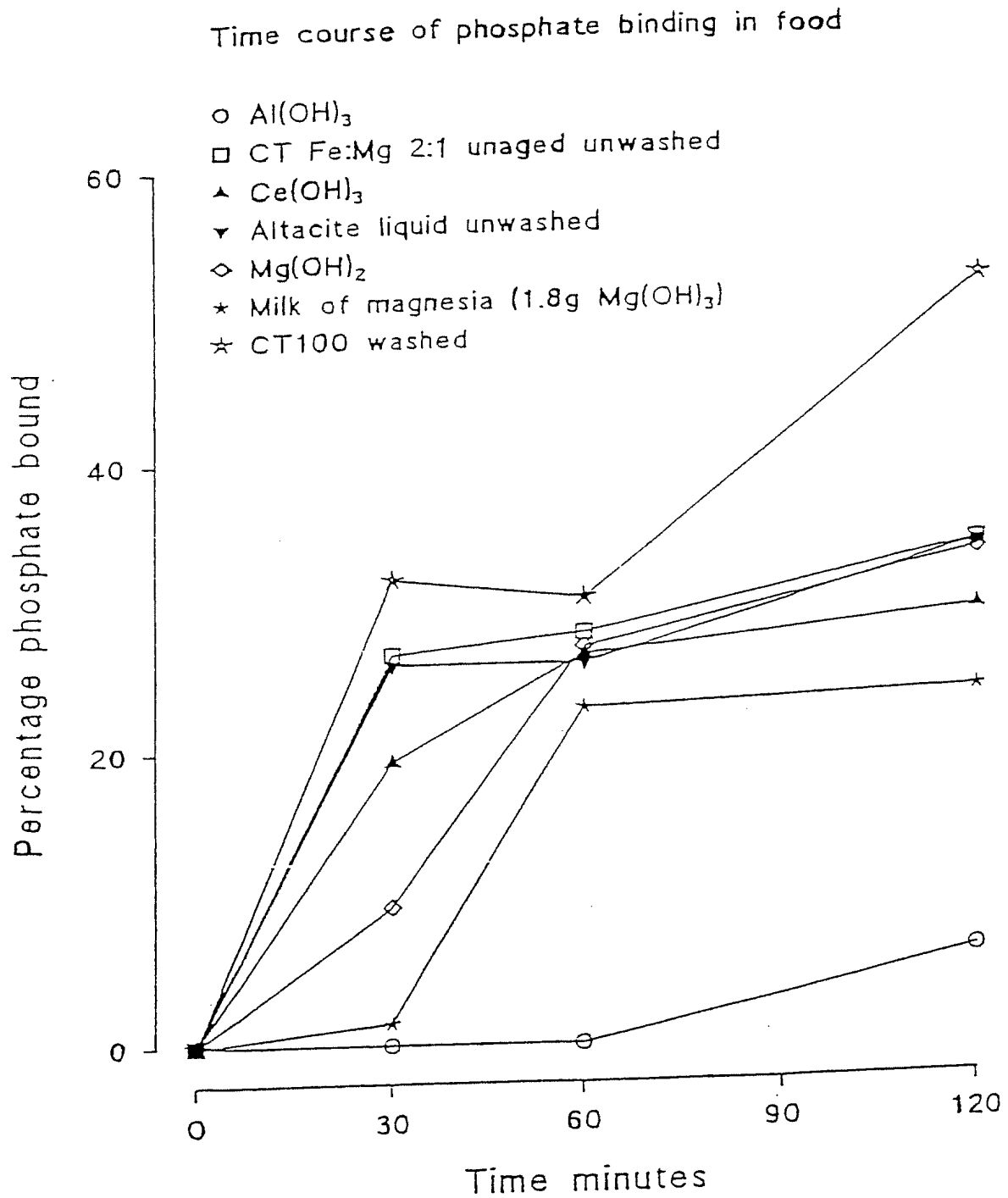
Effect of increasing weight of compound on percentage phosphate bound at pH7



SUBSTITUTE SHEET (RULE 26)

5/10

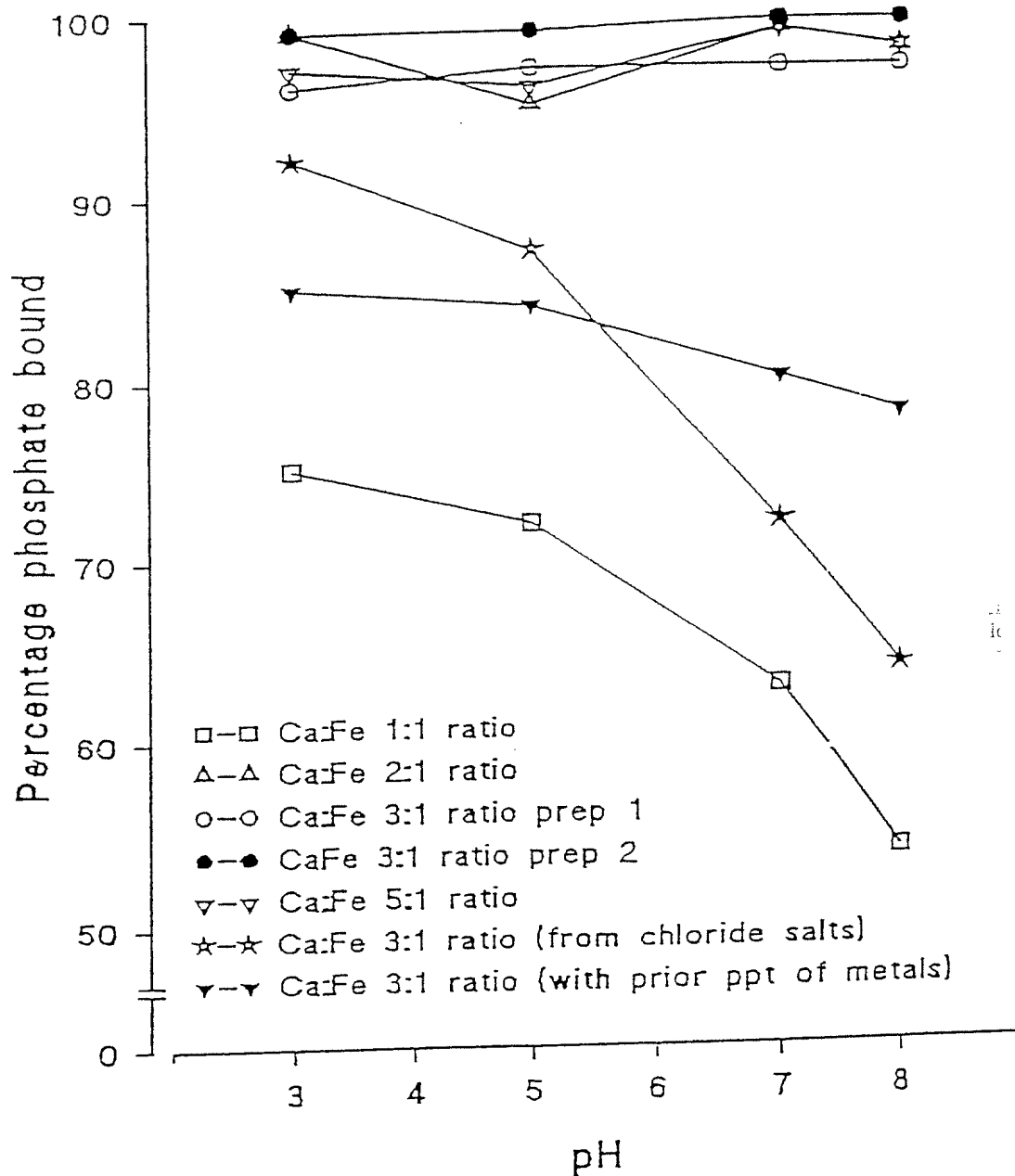
Figure 5:



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Figure 6:

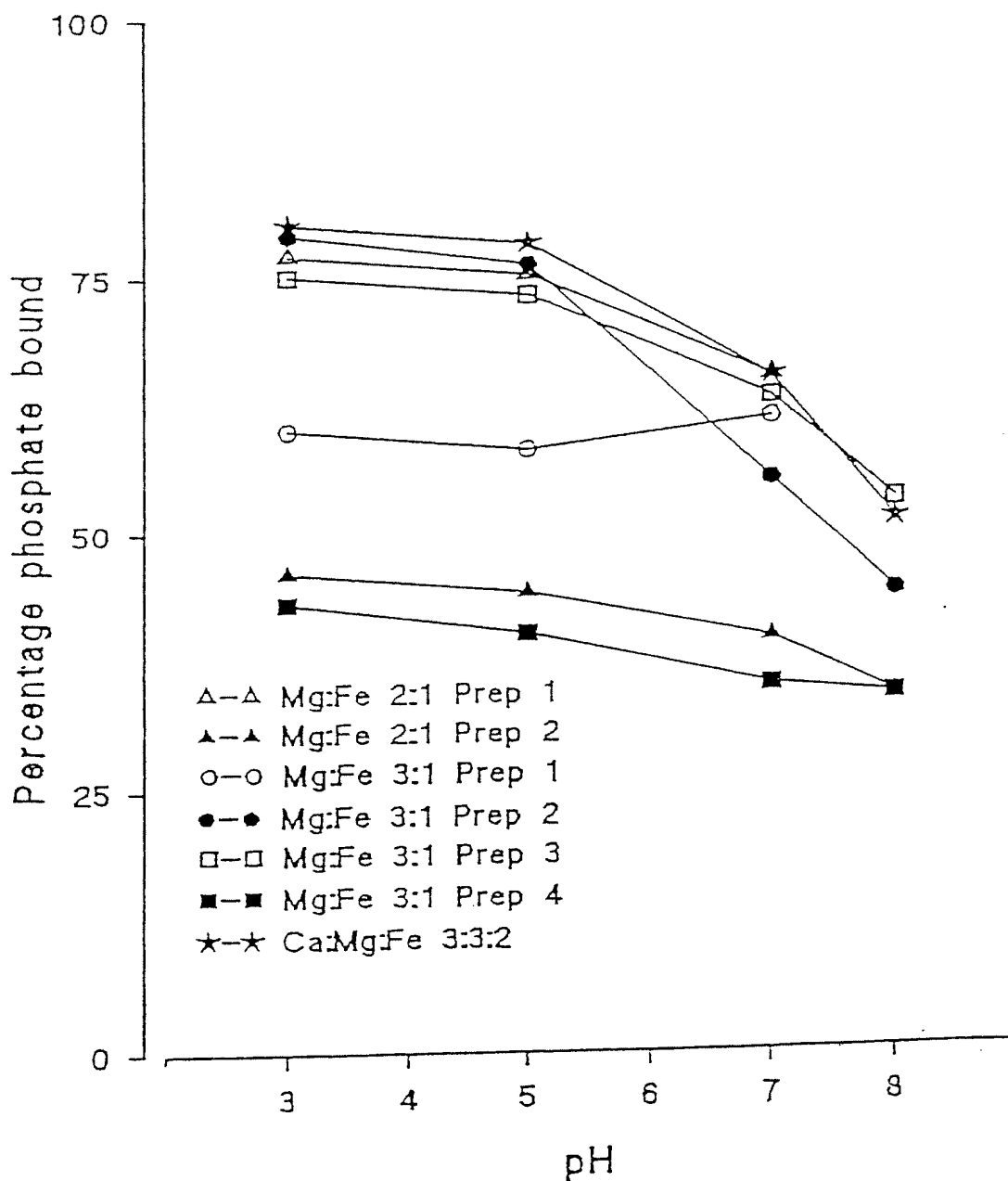
Phosphate binding by the calcium ferric iron preparations
over the pH range 3-8



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Figure 7:

Phosphate binding by the magnesium ferric iron and calcium magnesium ferric iron preparations over the pH range 3–8



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Figure 8:

Phosphate binding by aluminium hydroxide, magnesium hydroxide and calcium carbonate over the pH range 3-8

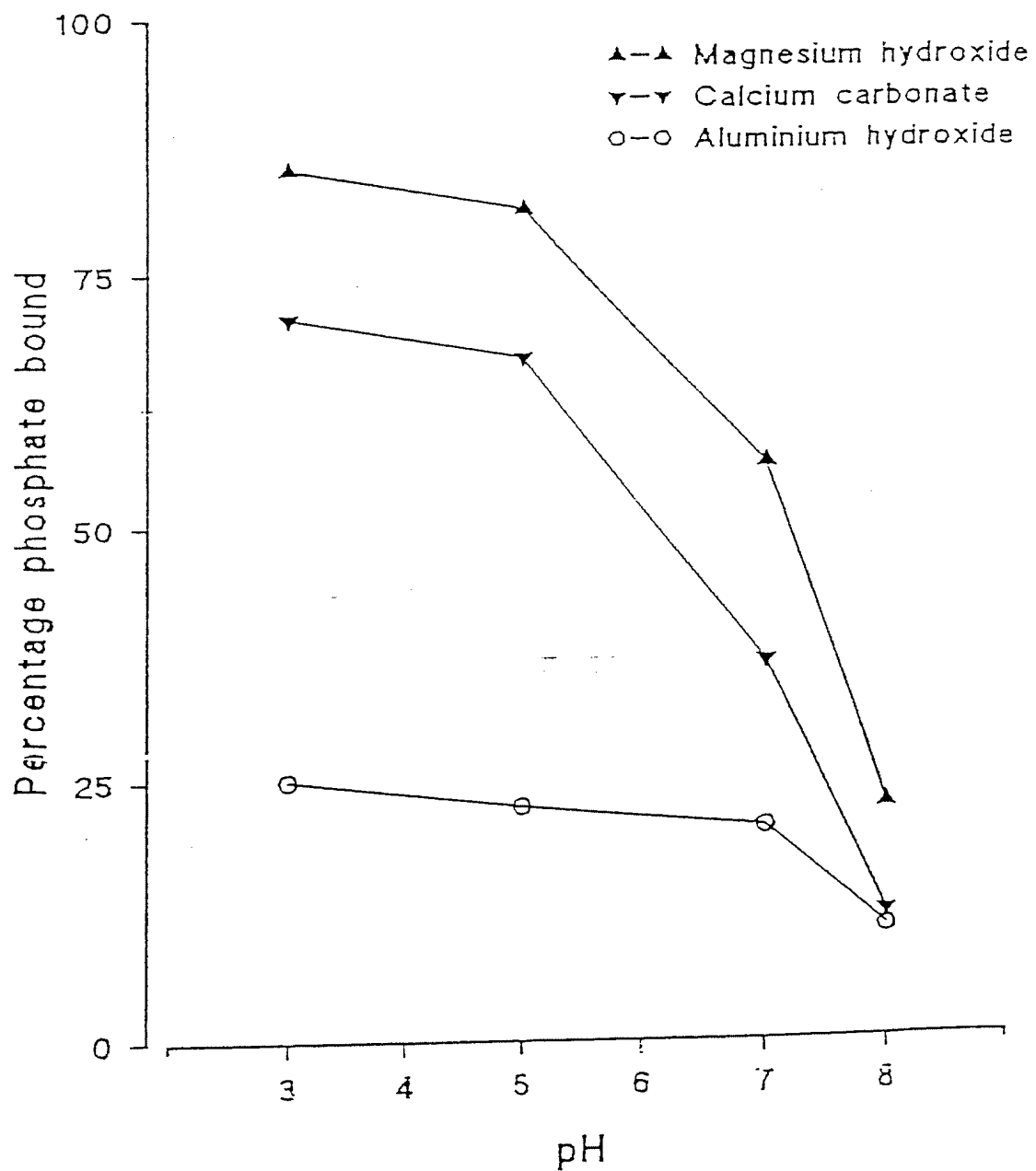
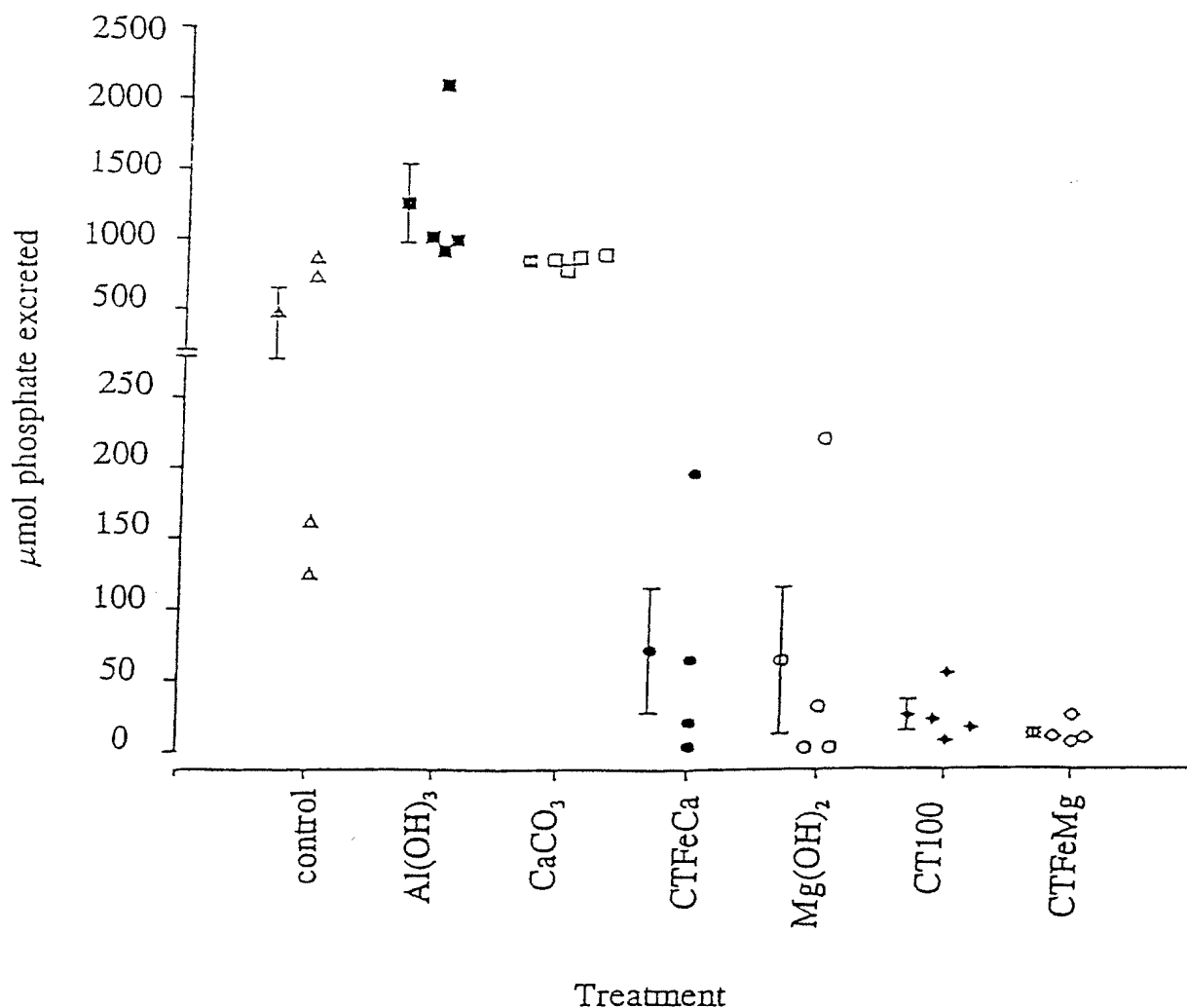


Figure 9: 9/10

Individual and mean (± 1 SEM) urinary phosphate excretion for control rats and those treated with phosphate binding compounds.

Individual values of urinary phosphate excretion ($\mu\text{mol}/24$ hours) were plotted for controls (Δ) and animals treated with $\text{Al}(\text{OH})_3$ (\blacksquare), CaCO_3 (\square), CTFeCa (\bullet), $\text{Mg}(\text{OH})_2$ (\circ), CT100 (\blacklozenge) and CTFeMg (\diamond). Mean (\pm SEM) for each group are presented by points with error bars. * $p < 0.05$ compared to $\text{Al}(\text{OH})_3$ treated animal groups.



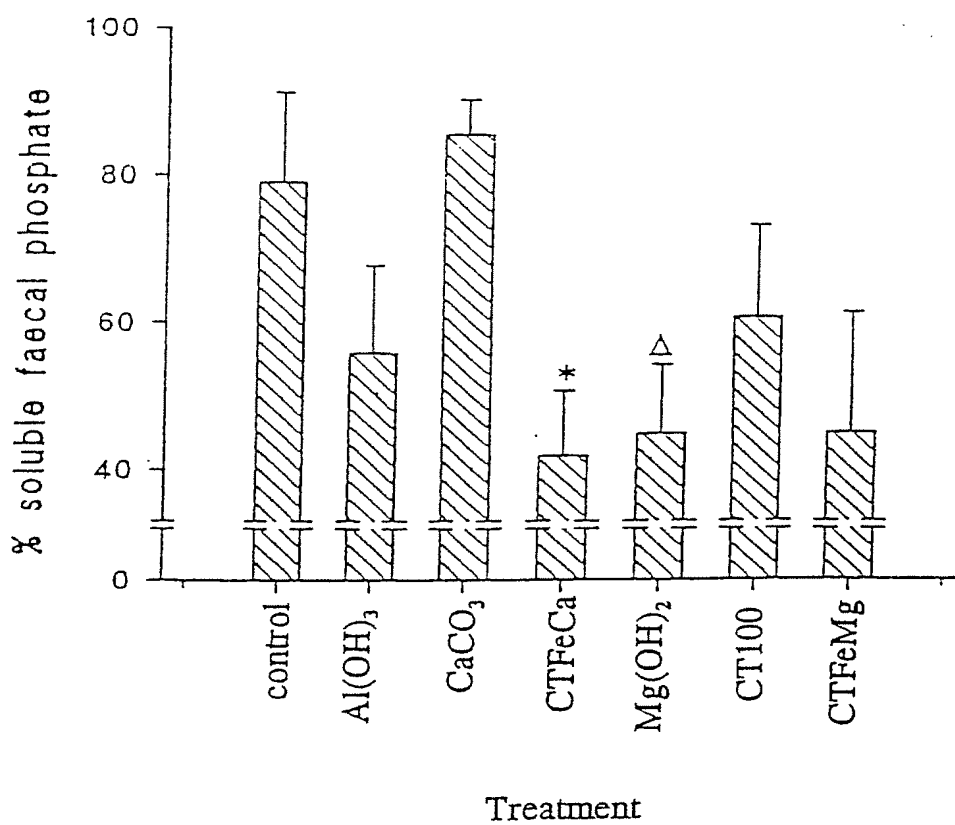
10/10

Figure 10

Mean (+1SEM) soluble faecal phosphate (g^{-1} dry weight as a percentage of total soluble and insoluble) faecal phosphate (g^{-1} dry weight) for control rats and those treated with phosphate binding compounds.

* $p < 0.05$ compared to control and CaCO_3 treated animals

$\Delta p < 0.05$ compared to CaCO_3 treated animals



INTERNATIONAL SEARCH REPORT

Inter- national Application No

PCT/GB 98/02834

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K33/26 A61K33/06 A61K33/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	DE 34 02 878 A (ALGINA AG) 1 August 1985 see page 4, paragraph 3 - page 5, paragraph 2; claims 1-3,6-9 see page 7, line 8, paragraph 2 see page 7, line 15, paragraph 3 see page 8, paragraph 4 see page 9, paragraph 4; example 1 --- -/--	1,2,5-7 4,12-15

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 January 1999

Date of mailing of the international search report

16/02/1999

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Authorized officer

Kanbier, D

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/02834

S CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 017, no. 551 (C-1117), 5 October 1993 & JP 05 155776 A (OTSUKA PHARMACEUT FACTORY INC), 22 June 1993 see abstract & DATABASE WPI Week 9329 Derwent Publications Ltd., London, GB; AN 93-232270 & JP 05 155776 A (OTSUKA SEIYAKU KOGYO KK) , 22 June 1993 see abstract ---	1,4
X	GB 2 254 556 A (FISONS PLC) 14 October 1992	1,2,5,6
A	see page 4, line 2-3; claims 1,7,44 see page 3, line 31-32 ---	4
X	US 5 654 011 A (JACKSON SHERRY D ET AL) 5 August 1997 see column 2, line 34-51; claim 1 ---	1,2,5,6
X	WO 97 22266 A (VIFOR INT AG) 26 June 1997 see claims 1-5 ---	1,7
X	PATENT ABSTRACTS OF JAPAN vol. 011, no. 371 (C-462), 3 December 1987 & JP 62 145024 A (ASAHI CHEM IND CO LTD), 29 June 1987	1
A	see abstract & DATABASE WPI Week 8731 Derwent Publications Ltd., London, GB; AN 87-218330 & JP 62 145024 A (ASAHI CHEM IND CO LTD) , 29 June 1987 see abstract ---	4-8, 12-14
X	US 5 571 336 A (WURZBURGER STEPHEN R ET AL) 5 November 1996 see column 2, line 8-15; claim 1 ---	12-15
X	DATABASE WPI Week 7832 Derwent Publications Ltd., London, GB; AN 78-58210a XP002091239 & SU 414 849 A (KHARK HOUSE BUILD) , 18 October 1977 see abstract ---	12-15
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02834

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUDAVARI ET AL, EDS.: "The Merck Index" 1996, MERCK & CO INC. XP002091238 see page 917, left-hand column, line 1-6 see page 331, right-hand column - page 332, left-hand column, line 4-7 see page 277, right-hand column ---	12-14
A	WO 96 30029 A (JOHNSON MATTHEY PLC) 3 October 1996 see page 1, line 10 - page 3, line 18; claims 1,6 ---	1,4,5,7, 8,12,13
A	PATENT ABSTRACTS OF JAPAN vol. 010, no. 194 (C-358), 8 July 1986 & JP 61 036222 A (CHUGAI PHARMACEUT CO LTD), 20 February 1986 see abstract -----	12-15

INTERNATIONAL SEARCH REPORT

I. national application No.

PCT/GB 98/ 02834

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-15
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-15
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02834

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3402878 A	01-08-1985	EP 0150792 A JP 60237020 A	07-08-1985 25-11-1985
GB 2254556 A	14-10-1992	NONE	
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WO 9722266 A	26-06-1997	DE 19547356 A AU 1374797 A EP 0868125 A NO 982722 A	26-06-1997 14-07-1997 07-10-1998 18-08-1998
US 5571336 A	05-11-1996	NONE	
WO 9630029 A	03-10-1996	AU 4951496 A BR 9607926 A CA 2216437 A CN 1184428 A CZ 9703017 A EP 0817639 A NO 974425 A PL 322450 A	16-10-1996 09-06-1998 03-10-1996 10-06-1998 15-07-1998 14-01-1998 24-11-1997 02-02-1998